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RESEARCH ARTICLE

TRANSFER AND MOLECULAR MAPPING OF *AEGILOPS TAUSCHII*-DERIVED HESSIAN FLY RESISTANCE GENES (*H22, H23, H24,* AND *H26)* FROM D GENOME OF *TRITICUM AESTIVUM* ONTO A GENOME CHROMOSOMES OF *TRITICUM TURGIDUM* BY INDUCED HOMOEOLOGOUS RECOMBINATION.

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Abstract

Aegilops tauschii Coss. (2n=14, DD) is a rich source of disease resistance genes for the improvement of cultivated wheat including several resistance genes against Hessian fly. To date, five Hessian fly resistance genes (*H13*, *H22*, *H23*, *H24*, and *H26*) have been transferred from *Ae*. *tauschii* to common wheat (*Triticum aestivum* L.). In this study, we attempted the transfer of four genes *H22* (1D), *H23* (6DS), *H24* (3DL), and *H26* (4D) from *T*. *aestivum* D genome onto A genome chromosomes of *T*. *turgidum*. The *T*. *aestivum* resistant parents WGRC01 (*H22* on 1D), WGRC03 (*H23* on 6DS), WGRC06 (*H24* on 3DL), and WGRC26 (*H26* on 4D) were crossed with *T*. *turgidum* cv. Langdon disomic substitution lines LDN 1D(1A), LDN 6D(6A), LDN 3D(3A), and LDN 4D(4A). We targeted the transfer of Hessian fly resistance genes into D-genome substitution chromosomes of *T*. *turgidum* by homologous recombination. In total 88 crosses were made. The resulting F_1 plants (345 seeds) were backcrossed with the LDN 5D(5B) substitution line in which chromosome 5B is absent and replaced by a pair of 5D chromosomes with the objective of transferring D genome Hessian fly resistance genes onto A or B genomes of *T*. *turgidum* by homoeologous recombination. A total of 2,053 segregating BC_1F_1 plants were tested for Hessian fly resistance, and the resistant plants (1,132) were backcrossed again with LND 5D(5B) to produce BC_2F_1 and selfed to produce BC_1F_2. In the BC_1F_1 populations, 24 families segregated for an excess of resistant plants than the expected 1:1 resistant to susceptible plants suggesting that they were putative A-D genome positive recombinants. Mapping analysis using microsatellites was used in these families to identify recombinants between A- and D- genome chromosomes. The data indicated that *H22* recombinants were recovered consisting of the distal part of the short arm of 1A, the proximal of 1DS, and the complete long arm of 1D. The recombinant can be described as T1AS-1DS·1DL.

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The recombinant involving *H23* probably consisted of the whole short arm of 6D and the long arm of 6A, and is described as T6DS·6AL. The centromeric marker indicated that this recombinant has the centromere from chromosome 6A. In addition, monosomic substitution lines were recovered for the remaining resistance genes *H24* and *H26*. These monosomic substitution lines are useful germplasm for further manipulation aimed at transferring genes *H24* and *H26* to durum wheat.

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Introduction:-

Each year infestations of the Hessian fly, *Mayetiola destructor* (Say), cause serious damage to both bread and durum wheat in many parts of the world. In the United States, the use of genetic resistance has protected common wheat for the last 50 years (Ratcliffe and Hatchett, 1997). Genetic resistance provides the most effective and efficient way to control this devastating insect. To date, 29 Hessian fly-resistance genes (*H1* through *H29*) have been identified in *Triticum*/*Aegilops* species and *Secale cereale* L. (McIntosh et al., 1998) and are being used in bread wheat improvement. The mechanism of resistance conditioned by these genes is by antibiosis, whereby the first instars die soon after they begin to feed on plants. A gene-for-gene relationship exists between the resistance in wheat and avirulence in the Hessian fly. Homozygous recessive pairs of genes (Hatchett and Gallun, 1970) condition virulence in the insect. Because of this highly specific interaction, 16 biotypes (designated Great Plains and A through O) have been isolated from field populations and are differentiated only by their ability or inability to survive on and stunt wheat with specific resistance genes.

Breeding for resistance to a wide range of disease and insect pests has been a major emphasis of most wheat improvement programs. The selection pressure from genetic resistance in modern agricultural systems has forced the pathogen and insect population to overcome the resistance, which becomes ineffective a few years after deployment. In these agricultural systems, the genetic variability is greatly reduced and makes wheat increasingly vulnerable to biological and environmental stresses. Fortunately, a large amount of genetic diversity exists in the wild relatives of cultivated wheat (Friebe et al., 1996).

Several approaches have been proposed for the production of wheat-alien chromosome translocations. Spontaneous wheat-alien translocations can arise from centromeric-breakage and fusion, which involves the misdivision of univalent chromosomes and the reunion of telocentric chromosome arms at the centromere. The transfer of small alien segments to wheat usually requires the use of induced homoeologous recombination and ionizing radiation. These two methods have been widely used to transfer novel resistance genes to wheat (Friebe et al., 1996).

A considerable number of successful alien introgressions involving homoeologous chromosome segments have been achieved in wheat (Friebe et al., 1996; Sears, 1993). Very little progress, however, has been made in durum wheat (Ceoloni et al., 1996; Luo et al., 1996) because the buffering ability of durum wheat is less than that of common wheat (Ceoloni et al., 1996; Joppa, 1993). Transfers from the A and B genomes of *Triticum aestivum* L. can be accomplished through homologous recombination, whereas transfers from the D genome or other wild relatives of wheat require enhanced pairing through induced homoeologous recombination by using *ph* mutant or genetic stocks where chromosome 5B is absent. Luo et al. (1996) used two cycles of homoeologous recombination to transfer the *Kna1* gene for enhanced tolerance to K^+/Na^+ from chromosome 4D of common wheat to 4B of durum wheat. After the first cycle, only terminal 4D/4B recombinants were recovered. Interstitial recombinants were recovered after the second cycle of recombination.

Only a few other successful transfers have been reported in durum wheat. Ceoloni et al. (1996) transferred the powdery mildew resistance gene *Pm13* from *Aegilops longissima* Schaseinf. & Muschl. into durum wheat, a gene previously transferred to common wheat. The same authors also transferred the leaf resistance-gene *Lr19* derived from *Agropyron elongatum* to chromosome 7A. Friebe et al. (1999) transferred the rye-derived, Hessian fly-resistance genes *H21* and *H25* from common wheat into durum wheat. These genes exist as translocations between wheat and rye chromosomes; *H21* is a Robertsonian translocation, T2BS·2RL, and *H25* is an intercalary translocation, Ti4AS·4AL-6RL-4AL.

Considerable attention is being given to *Aegilops tauschii* Coss. (2n=14, DD) as a source for wheat improvement with genes affecting a considerable number of characters including resistance to several pests and diseases (Cox, 1991; Gill and Raupp, 1987; Hatchett and Gill, 1981). To date, five Hessian fly-resistance genes have been transferred from *Ae. tauschii* into common wheat. *H13* is present in the wheat germplasm KS81H1640HF, *H22* was transferred to the wheat germplasm WRGC01, *H23* is present in the wheat germplasm WGRC03, *H24* is present in the wheat germplasm WGRC06, and *H26* is present in the resistant wheat germplasm WGRC26. These genes confer resistance to wide range of the insect biotypes, and may increase the durability of resistance if deployed. In the present study, we report on the transfer of these genes from hexaploid WGRC germplasm into durum wheat.

Material and Methods:-

Plant materials:-

The wheat germplasms WRGC01 (*H22*), WGRC03 (*H23*), WGRC06 (*H24*), and WGRC26 (*H26*); and the Langdon disomic substitution lines LDN 1D(1A), LDN 6D(6A), LDN 3D(3A), LDN 4D(4A), and LDN 5D(5B) (Joppa, 1993) used in this study are maintained by the Wheat Genetics Resource Center, Kansas State University, Manhattan, Kansas, USA. The resistant parents WGRC01, WGRC03, WGRC06, and WGRC26 were first crossed with Langdon disomic substitution lines LDN 1D(1A), LDN 6D(6A), LDN 3D(3A), and LDN 4D(4A), respectively (Figure 1). Eighty-eight different crosses were made between the germplasm lines and the Langdon disomic substitution lines to allow for homoeologous recombination between the A- and D- genome chromosomes. *H22*, present in WGRC01, was mapped to chromosome 1D; *H23*, which is present in WGRC03, was mapped to chromosome arm 6DS; *H24*, present in WGRC06, was mapped to chromosome arm 3DL; and *H26* transferred to WGRC26 was mapped to chromosome 4D. The F_1 generation plants are monosomic for one chromosome from the D genome (1D for LDN 1D(1A), 3D for LDN 3D(3A), 4D for LDN 4D(4A), and 6D for LDN 6D(6A)) and one chromosome from the A genome (1A for WRGC01, 6A for WGRC03, 3A for WRGC06, and 4A for WGRC26). Crossing these plants with LDN 5D(5B), which lacks *Ph* gene, will promote pairing between these two chromosomes and allow the transfer of Hessian fly-resistance genes from the D to the A genome.

Plants from these crosses were sown in 5x5 cm vermiculite-filled pots. Small seedlings were kept in vernalization at 10°C and 8-hour daylength for 7 weeks. The seedlings were transplanted into 3.5-l pots containing a 2:1:1 mixture of soil, peat, and Perlite. Plants were grown in a greenhouse at 15-25°C with supplemental lighting to provide a 16-hour day-length. The F_1 plants were backcrossed as females with the LDN 5D(5B) substitution line to allow for homoeologous recombination. The central florets of each spikelet were removed, and the spikelets were trimmed to just above the top of the stigma. Pollinations were made by the approach method. More than 2,000 segregating BC_1F_1 plants were tested for their reaction to Hessian fly and the resistant plants were backcrossed again with LDN 5D(5B) to produce a BC_2F_1 and selfed to produce BC_1F_2. In the BC_1F_1 populations, 24 families segregated for an excess of resistant plants than the expected 1:1 resistant to susceptible plants suggesting that they were probably putative A-D genome positive recombinants. In the case that the plants still have the whole D chromosome the expected ratio of resistant to susceptible plants will be less than 1:1 because of the transmission of the univalent chromosome during meiosis, which is around 25%.

Cytogenetic Analysis:-

Pollen mother cells (PMCs) were collected in the F_1 generation and each subsequent generation to study metaphase I pairing in the hybrids. Anthers were collected at appropriate stage, fixed in 3:1 absolute ethanol and glacial acetic acid for 1 week, and kept at 4° C. Fixed anthers were squashed in 1% acetocarmine and metaphase I pairing was recorded in at least 150 PMCs for each cross. Pictures were taken using a Zeiss photomicroscope. Chromosome numbers also were recorded in each generation. C-banding was according to Gill et al. (1991).

Microsatellite mapping:-

Microsatellites that mapped to distal region of A and D genome were selected from Röder et al. (1998). Specific D-genome primers also were selected from Pestsova et al. (2000). The primers were first tested in the parents and only polymorphic primers were tested in the population. The PCR reaction was in a 25µl volume composed of 1µl of template DNA, 2.5µl of 10X buffer, 2.5µl dNTPs (2.5 mM), 1.25µl MgCl2 (25 mM), 2µl of left and right primer, 0.25µl Taq, and 15.5µl ddH2O. Depending on the annealing temperature, the PCR program ran for 36 cycles at 94°C for 3 min, with a first cycle of 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min, and a final extension at 72°C for 10 min, and kept at 4°C. PCR products were separated on 2.3% Agarose (Metaphor) gels in 1X TBE buffer. Gels were stained with ethidium bromide, visualized with UV light, and photographed.

Hessian fly testing:-
Evaluation of resistance to Hessian fly was according to Hatchett et al. (1981) and Friebe et al. (1990). Plants in the seedling stage were evaluated for their reaction to biotype L of the Hessian fly, the most virulent naturally occurring biotype. Greenhouse temperatures were maintained between 18ºC and 24ºC throughout the tests. Adult Hessian flies were allowed to oviposit for 8 hours on plants in the one- to two-leaf stage. Plants were examined after oviposition, and all were found to be infested with large numbers of eggs on the first leaf. Susceptibility or resistance was determined 15 days after egg infestation. The susceptible plants are stunted and dark green, whereas the resistant plants are normal in color and were examined for dead larvae to confirm resistance.

Results:-

Chromosome number and metaphase I pairing:-
All the F_1 plants had 35 chromosomes and paired as 28 bivalents and 7 univalents, which corresponds to six D-genome and one A- genome chromosomes (1A, 3A, 4A and 6A) (Figure 2). The F_1 seeds were shriveled and some of did not germinate. In the following backcross generations, the chromosome number ranged from 28 to 40. After the first backcross, the male fertility of plants increased and more seeds were obtained.

Transfer of *H22* into durum wheat:-
The cross WGRC01/LDN 1D(1A) produced 90 seeds (Table 1). Sixteen of those were germinated and backcrossed with Langdon 5D(5B) producing 800 BC_1F_1 plants. Two planting dates were used for Hessian fly testing. In the first planting, 291 plants were evaluated for their resistance to the insect, and 16 were resistant. In the second planting, 385 plants were evaluated for their resistance and 248 plants were resistant. One hundred twelve BC_1F_2 and BC_2F_1 plants were selected for the next generation. The seed in the backcross generations were plump and red. These plants were backcrossed with LDN 5D(5B) to produce BC_3F_1 or selfed to produce BC_1F_3. All the BC_2F_2 and BC_1F_3 seeds were plump and white.

Five families segregated more than 3:1 for resistant and susceptible plants. These families are given in Table 2. The segregation ranged from 14:1 for family 0232 to 5:1 for family 0231. Plants from these families were grown and checked cytologically, and only plants with 2n=28 chromosomes were selected for the microsatellites mapping.

Transfer of *H23* into durum wheat:-
Thirty-three crosses of WGRC03/LDN 6D(6A) produced 165 seeds (Table 1). Fifty-two of those were germinated and backcrossed with Langdon 5D(5B), which produced 2,600 BC_1F_1 plants. Two planting dates were used for Hessian fly testing. In the first planting, 494 plants were evaluated for their resistance to the insect; 221 were resistant. In the second planting, 531 plants were evaluated for their resistance and 486 plants were resistant. Three hundred eighty-seven BC_1F_2 and BC_2F_1 plants were selected for the next generation. The seed in the backcross generations were plump and red. These plants were then backcrossed with LDN 5D(5B) to produce the BC_3F_1 and selfed to produce the BC_1F_3. All the BC_3F_1 and BC_1F_3 seeds were plump and white.

Two families from the first planting and 15 families from the second planting segregated more than 3 to 1 resistant to susceptible plants. These families are given in Table 3. The segregation ranged from 40:1 for families 0272 and 0279 to 5:1 for family 0252. Plants from these families were grown and checked cytologically. Only plants with 2n=28 chromosomes were selected for microsatellite mapping.

Transfer of *H24* into durum wheat:-
Seventeen crosses of WGRC06/LDN 3D(3A) produced 80 seeds (Table 1). Ten of those were germinated and backcrossed with Langdon 5D(5B) to produce 500 BC_1F_1 plants. Two planting dates were used for the Hessian fly testing. In the first planting, 157 plants were evaluated for their resistance to the insect, and six were resistant. In the second planting, 167 plants were evaluated for their resistance and 148 plants were resistant. Thirty-nine BC_1F_2 and BC_2F_1 plants were selected for the next generation. The seed in backcross generations was plump and red. These plants were then backcrossed with LDN 5D(5B) to produce BC_3F_1 and selfed to produce BC_1F_3. All the BC_3F_1 and BC_1F_3 seeds were plump and white.

Seven families from the second planting date segregated more than 3 to 1 resistant to susceptible plants. These families, given in Table 4, segregated from 17:1 for the families 0237 and 0241 to 7:1 for the families 0238 and 0243. Plants from these families were grown and checked cytologically. Only plants with 2n=28 chromosomes were selected for microsatellite mapping.

Transfer of *H26* into durum wheat:-
Twenty crosses of WGRC26/LDN 4D(4A) produced 10 seeds (Table 1). Only two of those were germinated and backcrossed with Langdon 5D(5B), which produced 40 BC_1F_1 plants. Most of the F_1 plants died and did not germinate. Two planting dates were used for Hessian fly testing. In the first planting, none of the plants were resistant. In the second planting, 28 plants were evaluated for their resistance and 25 plants were resistant. Six BC_1F_2 and BC_2F_1 plants were selected for the next generation. The seed in backcross generations were plump and red. These plants were then backcrossed with LDN 5D(5B) to produce BC_3F_1 and selfed to produce BC_1F_3. All the BC_3F_1 and BC_1F_3 seeds were plump and white.

One family, number 0244 from the second planting date, segregated 8:1 for resistant to susceptible plants (Table 5). Plants from this family were grown and checked cytologically. Only plants with 2n=28 chromosomes were selected for microsatellite mapping.

Microsatellite mapping:-
The microsatellite mapping for each gene will be treated separately. We used at least one primer for each chromosome arm of the A and D genomes to select the recombinants between the two genomes. Very distal markers were initially selected because recombination in distal part of the chromosome is higher than that in the proximal part as was shown previously for wheat (Gill and Gill, 1994). We used proximal primers to further characterize the recombinants.

Screening for *H22* recombinants:-
The Hessian fly-resistance gene *H22* is derived from WGRC01 and was mapped previously to chromosome 1D by Raupp et al. (1993). The markers used for screening for *H22* recombinants are listed in Table 6. Two families were selected among those that segregated more than 1:1 resistant to susceptible plants when tested for Hessian fly resistance (Table 2). All the plants within these families showed amplification with one or more of the microsatellite (WMS) primers previously mapped on chromosomes 1A and 1D.

The WMS GWM99 primer set, which mapped to 1AL, amplified one polymorphic allele of about 120 bp in the resistant parent WGRC01 and Langdon 5D(5B) (Figure 3a). We observed amplification in both parents because they all have chromosome 1A whereas in the case of LDN 1D(1A) no amplification was observed because this parent lacks chromosome 1A. No amplification was observed in any of the tested plants of both families suggesting that they lack the distal part of chromosome 1AL. The GWM232 primer set, which was mapped to 1DL, confirms this result. The primer GWM232 amplified as many as six alleles and only one allele of about 140 bp is believed to be informative (Figure 3d). All the plants in family 0226 showed the critical band suggesting that they have the distal part of chromosome arm 1DL. Except for plants No. 1 and No. 7 that lack this band in the family 0232, all the tested plants showed the informative allele. No amplification was obtained for these two plants. This result suggests that *H22* can be mapped precisely to chromosome arm 1DL. In order to check if the whole chromosome 1D was transferred to these putative recombinants, the GWM106 primer set, which mapped to distal part of chromosome arm 1DS was tested. The GWM106 primer set amplified one allele of about 120-150 bp (Figure 3b) in the parent Langdon 1D(1A). This allele was not amplified in either WGRC01 or Langdon 5D(5B). The actual size of GWM106 is around 81 bp (Röder et al., 1998). All the tested plants from both families 0226 and 0232 had a 120-bp band and, therefore, we can deduce that these plants have the distal part of chromosome arm 1DS. Another primer GDM33 was tested, which is specific to the D-genome and mapped to the very distal part of chromosome arm 1DS. All the tested the lines did not show the distal segment of chromosome arm 1DS (Figure 3e) indicating that these lines lack the very distal part of chromosome arm 1DS.

The last primer tested was GWM136 that was mapped to the distal part of chromosome arm 1AS. This marker amplified two alleles in the parents WGRC01 and Langdon 1D(1A) of about 300 bp and 400 bp for all tested individuals. The latter band was very intense in Langdon 5D(5B) (Figure 3c). For the first family 0226 all the plants showed the 1AS band and segregated for another smaller band. In the case of family 0232 plants No. 1 and No. 7 did not show any amplification, whereas the remaining plants showed the critical band of chromosome arm 1AS.

The mapping data indicate that all plants in the family 0226 and family 0232, except for plants 0232 #1 and 0232 # 7, have the distal segment derived from chromosome arm 1AS, the proximal arm of 1DS, and the long arm of chromosome 1D. These recombinants can be described as T1AS-1DS·1DL (Figure 3f). These results need to be confirmed later by using the genomic in situ hybridization (GISH) technique. By using total genomic DNA from

Aegilops tauschii as a probe and total genomic DNA from *T. turgidum* as blocking DNA this technique will allow us to visualize the D-genome chromatin that was transferred to A genome.

Screening for *H23* recombinants:-

The Hessian fly resistance gene *H23* is derived from WGRC03 and was mapped previously to chromosome 6D by Raupp et al. (1993). Later Ma et al. (1993) concluded that this gene is probably located in the short arm of chromosome 6D. The markers used for screening for *H23* recombinants are listed in Table 7. Eight families (0249, 0253, 0254, 0256, 0257, 0259, 0272, and 0279) were tested among those that segregated more than 3:1 resistant to susceptible plants when tested for Hessian fly resistance (Table 3). Most of the plants within these families showed amplification with one or more of the microsatellites primer sets investigated for chromosomes 6A and 6D.

The WMS GWM469 that was mapped to the distal part of chromosome 6DS amplified three polymorphic alleles in the parents WGRC03, the Langdon 6D(6A), and the Langdon 5D(5B) (Figure 4a). In the family 0254 all plants showed a similar amplified allele compared to the resistant parent WGRC03. Plant No. 4 had a very faint band. All the tested plants of the family 0272 showed the 6DS specific allele, except for plant No. 3 that had a missing band compared to the resistant parent. All the tested plants of family 0279 showed the resistant parent allele, except for plant No. 5, which had no amplification and therefore was missing the 6DS allele. All the following plants 0257 #1, #2, #3, #4, #5, #6, and #7 of the family 0257 showed a band as the resistant parent (Figure 4c, bottom) when using the 6DS specific primer. All the tested plants of family 0259 showed the same band, except for plant 0259 #3, which had a very faint band.

In order to precisely map the Hessian fly resistance gene to chromosome arm of 6DS and check if the plants show the allele of the opposite arm (6DL) of the resistant parent the following primer set GDM98 was tested. Primer GDM98 amplified one monomorphic allele in the resistant parent WGRC03, Langdon 6D(6A), and Langdon 5D(5B) (Figure 4b) of about 150 bp. None of the plants from the family 0254 showed this allele, suggesting that they do not have the distal segment of chromosome arm 6DL. Except for plants No. 3 and No. 5 in the family 0272, all the tested plants showed the resistant parent allele, suggesting that these plants had the complete 6D chromosome. Most of the tested plants of the family 0279 did not show the 6DL band, except for plants No. 2 and No. 3. These plants still had the complete 6D chromosome. In the remaining tested families 0257 and 0259, the following plants 0257 #1, #2, #3, #5, #6, and #7 showed a faint band similar to the parent Langdon 5D(5B). The plant 0257 #4 did not show any amplification, thus does not have the 6DL segment. The plants 0259 #1, #2, #3, #4, #5 of family 0259 showed the faint band similar to the 0279 population. Plants No. 6 and No. 7 did not have any amplification. Only plant No. 5 showed the band of the resistant parent WGRC03 (Figure 4b).

Additional primer sets that mapped to chromosome arms 6AS and 6AL were tested (Table 7). These primers were tested to determine whether or not the putative recombinants still retain parts of chromosome 6A. The WMS primer GWM459 mapped to 6AS and amplified as many as three alleles, whereas the WMS primer GWM334 amplified one monomorphic allele of about 114 bp (Figure 4d). Not all the plants of the family 0254 showed that specific band when tested by these primers, suggesting that they do not have the distal segment of 6AS. The families 0272 and 0279 showed contrasting results for these two primers, and only plants 0272 #3, 0279 #6, 0279 #7 and 0279 #8 did not show the 6AS specific band. Not all the plants from the both families 0257 and 0259 showed the 6AS band with GWM459.

In order to test whether or not the plants from the selected families have the 6AL specific allele the two primers GWM169 and GWM427 were tested. All the tested plants in the families 0254, 0257, and 0259 showed the 6AL allele when screened by the primer set GWM169 (Figure 4c). For the families 0272 and 0279, the plants 0272 #3, 0279 #1, and 0279 #5 did not show the 6AL allele. The last tested primer GWM427 amplified one polymorphic band in both parents WGRC03 and Langdon 5D(5B) (Figure 4d). Similar results were obtained for all tested families as for the previous primer set GWM169.

In summary for the *H23,* recombinants all the plants in the family 0254 are true recombinants. All plants in the family 0257, except plant No. 4, and plants No. 1, 2, 4, and 5 in the family 0259 have complete chromosome 6A and 6D, therefore, they are monosomic substitution lines. Plant 0259 #7 in the family 0259 and three plants (No. 6, 7, and 8) in the family 0279 are recombinants. Also in some families, there are plants that have complete chromosome 6A and telocentric chromosome for either 6DS or 6DL. The recovered recombinants can be described as T6DS/6AL. By using very proximal markers for both chromosome arms 6DS and 6AL, we can verify the

localization of the crossing-over event. To check the localization of the breakpoints four primers were selected (Table 7). The GDM14, mapped to proximal part of chromosome arm 6DS, amplified two alleles. All the tested recombinants (0254 #2, 0254 #3, 0254 #4, 0259 #7, 0279 #6, 0279 #7, and 0279 #8) had the 6DS segment (Figure 4e). Then GDM132, mapped to very distal part of 6DS, was tested in these recombinants. The results in Figure 4e indicate that all recombinants had the distal segment from chromosome arm 6DS. Two more specific primers for 6A chromosome were tested; the primer GWM570 that mapped to proximal part of 6AL indicated that all the recombinants have this corresponding segment. The primer GWM494 that mapped to centromeric region of chromosome 6A indicated that all the tested recombinants had the centromere of chromosome 6A, therefore, they can be presented as T6DS·6AL (Figure 4f).

These recombinants need to be confirmed later by using the GISH technique. By using this technique, we will be able to precisely map the localization of the crossing-over event.

Screening for *H24* recombinants:-

The Hessian fly resistance gene *H24* is derived from WGRC06 and was mapped previously to chromosome arm 3DL by Raupp et al. (1993). The wheat microsatellite markers mapped to distal part of chromosomes 3A and 3D were used for screening for *H24* recombinants (Table 8). The following families 0236, 0237, 0238, 0239, and 0241 that segregated more than 3:1 resistant to susceptible plants were selected for further characterization.

The wheat microsatellite GWM3 and GWM314 that mapped to the distal part of chromosome arm 3DL were tested first. The primer GWM314 amplified as many as three alleles while GWM3 amplified only one polymorphic allele (Figures 5a and 5b). For the family 0236, only plants No. 5 and No. 6 showed the band of the resistant parent. Most of the tested plants of family 0237 showed the resistant parent allele, except for plants No. 2 and No. 5. Similar results were obtained for families 0238, 0239 and 0241 where all the tested plants showed the 3DL specific allele when tested by both WMS primers.

The primers GWM161 and GWM183 that mapped to 3DS were then tested. The GWM161 amplified one monomorphic allele in both parents WGRC06 and Langdon 3D(3A) (Figures 5e and 5g). The plants 0236 #1, #2, #3, and #4 did not show the resistant parent allele, whereas plants of this same family that showed previously 3DL allele, had the 3DS band (plants # 5 and #6), suggesting that these two plants retained the complete chromosome 3D. All the plants in the following families 0238 and 0239 showed the 3DS segment, thus, they still retain the complete chromosome 3D. For the family 0237, only plant No. 1 did not show the 3DS allele, and in the family 0241 no amplification was obtained for the plants 0241 #1 and 0241 #3.

In order to check if the positive recombinants still retain the chromosome 3A the GWM369, that mapped to 3AS, was tested and amplified three different fragments in the resistant parent WGRC06, Langdon 3D(3A), and Langdon 5D(5B) (Figure 5f). Plants in family 0236 segregated for two alleles, plant 0236 #1 showed the band with smaller size, whereas plants 0236 #2, #3, #4, and #5 showed the upper band (Figure 5f), and plant 0236 #6 had both bands. All the plants in the family 0238 had the 3AS allele, whereas only plants No. 1, 5, and 6 in family 0239 had the same allele. The majority of plants in both families 0237 and 0241 had the specific allele for chromosome arm 3AS, except plants 0237 #1, 0237 #6, 0241 #1 and 0241 #3 (Figures 5e and 5f).

The WMS primers tested for the long arm of chromosome 3A were GWM162 and GWM391. The primer GWM391 amplified as many as three alleles in the family 0241 and five plants out of six showed the 3AL segment (Figure 5c). All the plants in family 0237, except No. 1, had the specific allele. Except plant No. 1 in family 0236, all the other plants showed the 3AL specific band, whereas in the other two remaining families 0238 and 0239 none of the plants have the distal segment of chromosome arm 3AL.

The WSM primer GWM162 showed that none of the plants tested in families 0237, 0238, 0239, and 0241 had the specific band that correspond to the GWM162 allele, and thus, these plants do not have the distal segment of chromosome arm 3AL (Figure 5d). All the checked plants of family 0236 had the 3AL segment, except for plant number 1.

In summary, in the family 0236 the plant No. 6 has a complete chromosome 3A and 3D, and thus it is a monosomic substitution. Plants No. 2, 3, 4 have a ditelocentric 3AL, plant No. 5 had a complete chromosome 3D and telocentric 3AL, and plant No. 1 is normal durum wheat. In the family 0237, plants No. 3, 4, 6, 7, and 8 have complete

chromosome 3A and 3D, therefore they are monosomic substitution lines, plant No. 1 is a disomic substitution line, and plants No. 2 and No. 5 have complete 3A plus a telocentric chromosome 3DS. All the plants in the family 0238 have complete chromosome 3D and telocentric chromosome 3AS. Plants No. 2 and 3 in family 0239 are disomic substitution lines (3D(3A)), whereas plants No. 1, 5 and 6 have complete chromosome 3D and telocentric 3AS. In the last family 0241 plants No. 2, 4, 6, and 7 are monosomic substitution lines where one chromosome of 3D is substituted for chromosome 3A. The remaining plants No. 1 and 3 have telocentric for long arms of both chromosomes 3A and 3D.

Screening for *H26* recombinants:-

The Hessian fly resistance gene *H26* is derived from WGRC26 and was mapped to chromosome 4D by Cox and Hatchett (1994). The wheat microsatellite markers used for screening for *H26* recombinants are listed in Table 9. Röder et al. (1998) mapped no WMS primers in the short arm of chromosome 4 D; therefore, a specific D-genome primer GDM129 was selected from Pestsova et al. (2000). Only one family was available for recombinant analysis in the case of the Hessian fly resistance gene *H26*.

The WMS GWM609 primer set, mapped to chromosome arm 4DL, amplified four polymorphic alleles in the resistant parent WGRC26, Langdon 4D(4A), and Langdon 5D(5B) (Figure 6b). The plants 0244 #1, #3, #5, and #7 showed the segment of the resistant parent, whereas 0244 #4 and #6 did not have the amplified fragment. The D-genome specific primer set GDM129 was tested to see which plants from this family possess the resistant parent allele. This primer was mapped to 4DS and amplified three polymorphic alleles in the parent WGRC26, Langdon 4D(4A), and Langdon 5D(5B) (Figure 6b). The plants 0244 #1, #3, #5, and #7 showed the amplified fragment of the resistant parent WGRC26. These are the same plants that showed the 4DL fragment; therefore, they still retain the whole chromosome 4D.

All the plants in the family 0244 showed the critical fragment with primer GWM601, mapped to 4AS, except plant No. 6 where there is less amplification (Figure 6a). The GWM160 primer mapped to 4AL was tested to see if the plants still have the other arm of chromosome 4A. This later primer amplified four polymorphic alleles in the WGRC26, Langdon 4D(4A), and Langdon 5D(5B) (Figure 6b). The samples were run in the polyacrylamide gel because the resolution was low in the Agarose gel. The results in Figure 6c indicates that the plants 0244 #1, 3, 5, and 7 have the 4AL segment.

In conclusion plants No. 1, 3, 5, and 7 have complete chromosomes 4A and 4D; therefore they are monosomic substitution plants. Plant No. 4 is normal durum wheat and plant No. 6 has a telocentric chromosome 4AL.

Discussion:-

Wild relatives and related species are important sources for disease and pest resistance for cultivated common and durum wheat. Several useful genes have been transferred to wheat by irradiation and homoeologous recombination, but only few have made contribution in cultivar development because of the non-compensating nature of transfers (Friebe et al., 1996).

To date, very few wheat-alien translocations have been transferred to durum wheat. Rao (1978) transferred a stem rust-resistance gene from rye and *A. elongatum* to durum wheat. The transmission of the translocation chromosomes through pollen was low and homozygous translocation lines were not recovered. Friebe et al. (1987; 1993), and Mujeeb-Kazi et al. (1996) transferred the wheat-rye translocation T1BL·1RS from common wheat to durum wheat. Ceoloni et al. (1996) transferred *Pm13* from *A. longissima* to chromosome 3B of durum wheat in the form of the translocation T3BL·3BS-3S^{l} and T3DL·3DS-3S^{l}.The same authors transferred the leaf rust-resistance gene *Lr19* from *A. elongatum* into durum wheat in the form of the translocation T7AS-7AeS·7AeL. The translocation chromosome is not transmitted through the pollen, thus preventing the recovery of homozygous lines. Later Luo et al. (1996) transferred *Kna1* gene conditioning enhanced tolerance to K^{+}/Na^{+} salt tolerance from chromosome 4D of common wheat to chromosome 4B of durum wheat.

This is the second report of resistance gene transfers from the D-genome of common wheat into A or B genomes of durum wheat using induced homoeologous recombination. Previously, Okamoto and Sears (1962) observed a certain degree of preferential pairing between A and D genomes of wheat. They reported that most homoeologous recombination occurred between these two genomes. Later, Jauhar et al. (1991) showed that strong preferential pairing occurred between chromosomes of the A and D genomes in the presence of the *ph1b* allele; 80% of the

pairing was between these two genomes. To transfer genes from the D genome of common wheat, the D-genome chromosome substitution lines in durum wheat cv. Langdon can be used. In double monosomic lines (one monosome is a D-genome chromosome), induced homoeologous recombination can in fact preferentially involve the two homoeologous chromosomes present in single dose. The same results were expected in our case where the F_1 plants derived from crosses between WGRC germplasm and Langdon disomic substitution lines were crossed with Langdon 5D(5B), where chromosome 5B carrying the *Ph* gene is absent and thereby enhancing homoeologous recombination.

During the transfer of these D-genome derived, Hessian fly-resistance genes, the resulting F_1 seeds were very shriveled and had a very low germination rate. Some germinated seeds died soon after because of high-level leaf chlorosis. Similar results were reported by Friebe et al. (1999) when transferring Hessian fly resistance genes from common wheat into durum wheat. Cooler temperatures in the greenhouse during autumn helped alleviate the problem of leaf chlorosis and increased plant survival.

Meiotic metaphase I pairing was analyzed in the F_1 hybrids of T. aestivum and T. turgidum. The chromosomes paired as 14 bivalents involving the A- and B- genome chromosomes plus seven univalents representing the D-genome chromosomes and confirmed the hybrid status of the F_1.

Putative recombinants between A- and D- genome chromosomes were selected using microsatellite (SSR) analysis. These markers show a much higher level of polymorphism in wheat as compared to other types of markers such as RFLPs, which show a very low level of polymorphism (Röder et al., 1998). Microsatellites currently are being used in gene mapping, assessing of genetic diversity, and testing the authenticity of cultivars. The availability of a high-density microsatellite map of wheat (Röder et al., 1998) and *A. tauschii* (Pestsova et al., 2000) makes these markers the first choice for gene mapping.

The microsatellite data for the *H22* transfer indicate that all plants in the family 0226 have the T1AS-1DS·1DL translocation. In the family 0232, the plants #2, #3, #4, #5, #6, and #8 have the T1AS-1DS·1DL translocation and two plants did not show any amplification. In the case of Hessian fly-resistance gene *H23*, all the plants in family 0254; plant #7 in the family 0259; and plants number 6, 7 and 8 from the family 0279 are true recombinants. These recombinants can be described as T6DS·6AL. For the Hessian fly-resistance gene *H24*, most of the plants still have complete chromosomes 3A and 3D and are monosomic substitutions. Some plants are disomic substitution lines 3D(3A), such as plants #2 and #3 in family 0239. In the case of the Hessian fly-resistance gene *H26*, plants #1, #3, #5, and #7 have complete chromosome 4A and 4D, and are monosomic substitution lines. Plant #4 is normal durum wheat and plant #6 has a telocentric chromosome 4AL.

The microsatellite analysis identified two recombinants T1AS-1DS·1DL with *H22* and T6DS·6AL with *H23*. Some plants in different families still have complete chromosomes from the D genome that conferred resistance to the Hessian fly. The high ratio of resistant plants observed during the Hessian fly testing indicates that the transmission of the D-genome chromosome is high. Monosomic substitution lines are useful germplasm that can be used for further isolation of recombinants between A- and D- chromosomes. These lines can be either crossed with *ph* mutant lines or selfed to select Robertsonian translocations. Similar results were observed by Luo et al. (1996) when attempting the transfer of the *Kna1* gene conditioning enhanced tolerance to K^+/Na^+ salt tolerance from chromosome 4D of common wheat to chromosome 4B of durum wheat. To successfully isolate recombinants between the A and D genomes another cycle of induced homoeologous recombination is necessary for the resistance genes *H24* and *H26* where transfers were not successful.

The data presented allowed precise mapping of *H22* and *H23* that previously only have been allocated to a whole D-genome chromosome. In the present study, *H22* was mapped to the long arm of chromosome 1D and *H23* was mapped to 6DS.

Durum wheat is a species traditionally of great importance for countries of the Mediterranean basin. However, it is severely damaged by the Hessian fly infestation every year. Providing these new germplasms will offer a broader genetic base for breeding durum wheat cultivars with genetic resistance that will provide protection against the Hessian fly.

Acknowledgements:-

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Table 1:- Number of crosses, and Hessian fly screening in derived progenies produced to transfer *Aegilops tauschii*–derived Hessian fly-resistance genes from *T. aestivum* onto *T. turgidum*.

Gene	No. cross.	F_1 seeds	No. Germi-nated F_1'S	No. BC_1F_1	Tested BC_1F_1	Resist-ant BC_2F_1& BC_1F_2	Select-ed BC_2F_1 & BC_1F_2	Proge-ny BC_2F_2 & BC_1F_3
H22	18	90	16	800	676	264	112	3,118
H23	33	165	52	2,600	1,025	707	387	12,680
H24	17	80	10	500	324	154	39	1,080
H26	20	10	02	40	28	25	06	19
Total	88	345	80	3,940	2,053	1,132	544	16,897

Table 2:-Families that deviated from the 1R:1S ratio for the Hessian fly resistance gene *H22*.

Family	Segregation (R:S)	Ratio (R:S)
0226	34:3	11:1
0229	28:5	6:1
0230	35:4	9:1
0231	26:5	5:1
0232	27:2	14:1
Total	150:19	8:1

Table 3:-Families that deviated from the 1R:1S ratio for the Hessian fly resistance gene *H23*.

Family	Segregation (R:S)	Ratio (R:S)
0249	27:1	27:1
0252	20:4	5:1
0253	25:0	25:0
0254	40:0	40:0
0256	30:1	30:1
0257	34:0	34:0
0259	24:0	24:0
0261	26:0	26:0
0267	16:2	8:1
0271	20:1	20:1
0272	40:1	40:1
0279	40:1	40:1
0284	17:0	17:0
0286	13:1	13:1
0288	10:0	10:0
Total	382:12	32:1

Table 4:-Families that deviated from the 1R:1S ratio for the Hessian fly resistance gene *H24*.

Family	Segregation (R:S)	Ratio (R:S)
0235	17:2	8.5:1
0236	26:1	26:1
0237	17:1	17:1
0238	21:3	7:1
0239	12:1	12:1
0241	34:2	17:1
0243	21:3	7:1

Total	148:13	11:1

Table 5:-Families that deviated from the 1R:1S ratio for the Hessian fly resistance gene *H26*.

Family	Segregation (R:S)	Ratio (R:S)
0244	25:3	8:1
Total	25:3	8:1

Table 6:-Primers used for Microsatellite mapping of Hessian fly-resistance gene *H22*.

Primer	Annealing temperature (^oC)	Chromosome arm location	Size (bp)
GWM99	60	1AL	117-120
GWM106	60	1DS	81
GWM136	60	1AS	278-321
GWM232	55	1DL	140-144
GDM 33	60	1DS	146-166

GWM: the lab designation of Gatersleben Wheat Microsatellite (Röder et al., 1998).
GDM: the lab designation of Gatersleben D-genome Microsatellite (Pestsova et al., 2000).

Table 7:-Primers used for Microsatellite mapping of Hessian fly-resistance gene *H23*.

Primer	Annealing temperature (^oC)	Chromosome arm location	Size (bp)
GDM98	60	6DL	146-153
GWM169	60	6AL	193-220
GWM334	50	6AS	110-114
GWM427	50	6AL	184-195
GWM459	55	6AS	118-126
GWM469	60	6DS	170-172
GDM14	60	6DS	134-138
GDM132	60	6DS	143-164
GWM494	60	6A	194-196
GWM570	60	6AL	143-149

Table 8:-Primers used for Microsatellite mapping of Hessian fly-resistance gene *H24*.

Primer	Annealing temperature (^oC)	Chromosome arm location	Size (bp)
GWM3	55	3DL	84
GWM161	60	3DS	145-154
GWM162	60	3AL	202-208
GWM183	55	3DS	105
GWM314	55	3DL	171-182
GWM369	60	3AS	184
GWM391	55	3AL	148

Table 9:-Primers used for Microsatellite mapping of Hessian fly-resistance gene *H26*.

Primer	Annealing temperature (^oC)	Chromosome arm location	Size (bp)
GDM129	60	4DS	116-120
GWM160	60	4AL	184-196
GWM601	60	4AS	142-152
GWM609	50	4DL	129

Figure 1:-Crossing scheme for transferring Hessian fly genes (the gene H22 is used as an example)

WGRC 01 X LDN 1D(1A)
2n=42 2n=28 [1D(1A)]
F_1: 13" +1'D^R + 1'D + 1'A + 6'(D)
F_1 X LDN 5D(5B)
2n=35 2n=28
BC_1F_1: 12"+ 1'(1A) + 1'(5B) + 1'($1D^R$) + 1'(5D)
(2n=28)
BC_1F_1 X LDN 5D(5B)
2n=28 2n=28
BC_2F_1: 12"+ 1'(1A) + 1'($1D^R$) + 1"(5D)

Figure 2:-Pairing analysis of the hybrid chromosomes at metaphase I

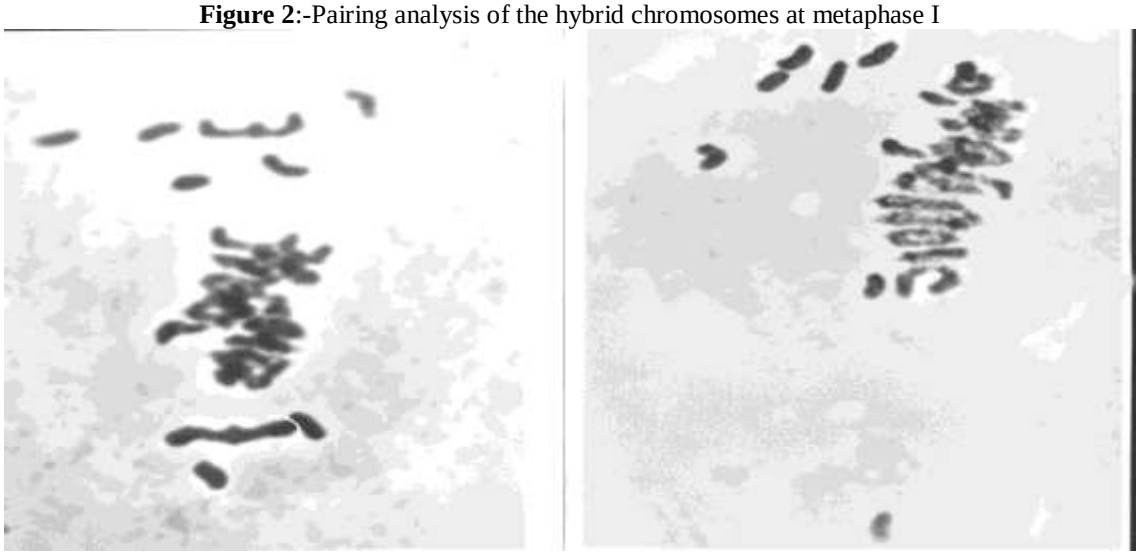


Figure 3:- Microsatellite mapping of the recombinants for the Hessian fly resistance gene *H22*.

Figure 3a:-Mapping of the microsatellite marker GWM99 (1AL, 120bp) on families 0226 and 0232. Top, M: marker, lane 1: WGCR01, lane 2: LND 1D(1A), lane 3: LND 5D(5B), lane 4 through lane 11: plants from family 0226. Bottom, M: marker, lane 1: WGCR01, lane 2: LND 1D(1A), lane 3: LND 5D(5B), lane 4 through lane 11: plants from family 0232.

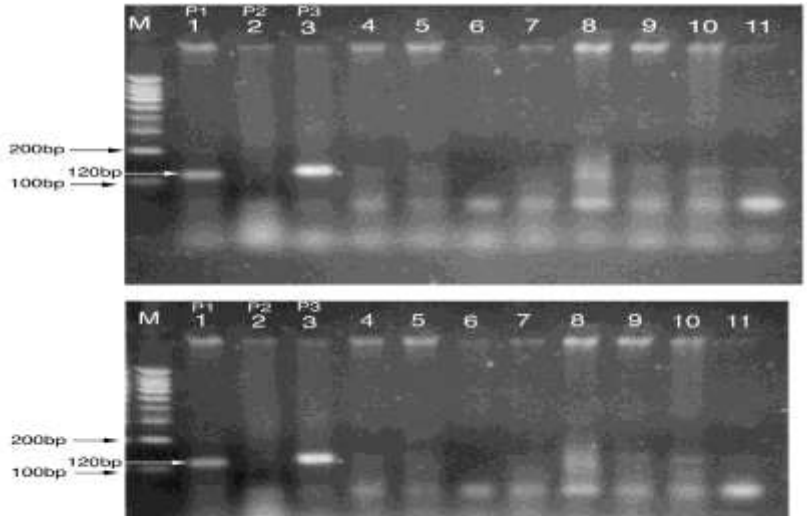


Figure 3b:-Mapping of the microsatellite marker GWM106 (1DS, 120bp) on families 0226 and 0232. Top, M: marker, lane 1: WGCR01, lane 2: LND 1D(1A), lane 3: LND 5D(5B), lane 4 through lane 11: plants from family 0226. Bottom, M: marker, lane 1: WGCR01, lane 2: LND 1D(1A), lane 3: LND 5D(5B), lane 4 through lane 11: plants from family 0232.

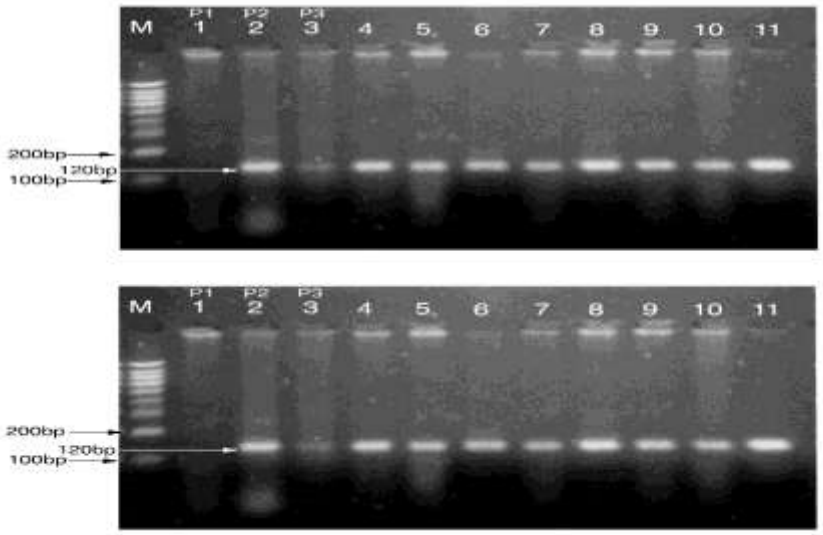


Figure 3c:-Mapping of the microsatellite marker GWM136 (1AS, 380bp) on families 0226 and 0232. Top, M: marker, lane 1: WGCR01, lane 2: LND 1D(1A), lane 3: LND 5D(5B), lane 4 through lane 11: plants from family 0226. Bottom, M: marker, lane 1: WGCR01, lane 2: LND 1D(1A), lane 3: LND 5D(5B), lane 4 through lane 11: plants from family 0232.

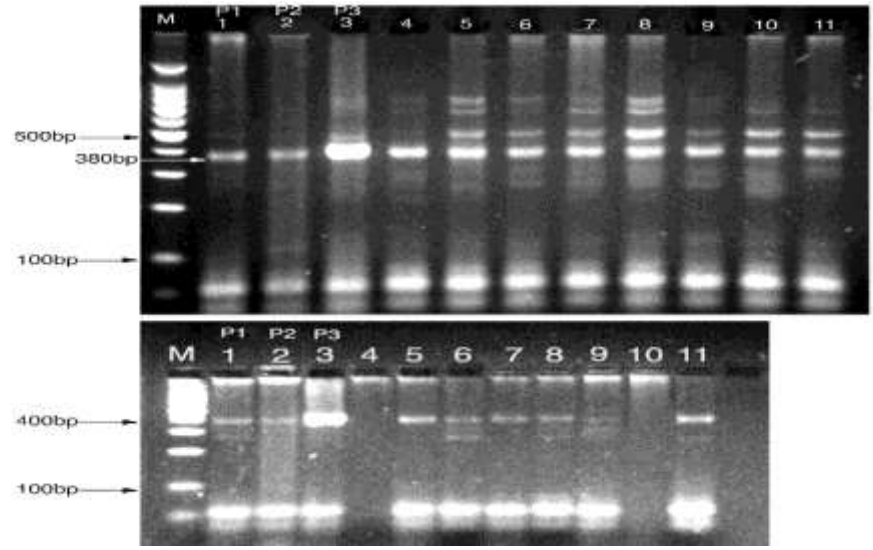


Figure 3d:-Mapping of the microsatellite marker GWM232 (1DL, 140bp) on families 0226 and 0232. Top, M: marker, lane 1: WGCR01, lane 2: LND 1D(1A), lane 3: LND 5D(5B), lane 4 through lane 11: plants from family 0226. Bottom, M: marker, lane 1: WGCR01, lane 2: LND 1D(1A), lane 3: LND 5D(5B), lane 4 through lane 11: plants from family 0232.

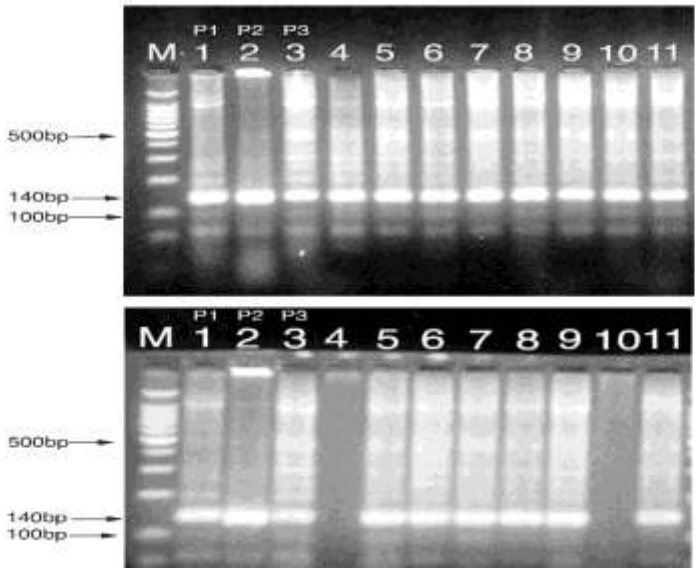


Figure 3e:-Mapping of the microsatellite marker GDM33 (1DS, 166 bp) and GWM106 on families 0226 and 0232. Top, M: marker, lane 1: WGCR01, lane 2: LND 1D(1A), lane 3: LND 5D(5B), lane 4 through lane 11: plants from family 0226. Bottom, M: marker, lane 1: WGCR01, lane 2: LND 1D(1A), lane 3: LND 5D(5B), lane 4 through lane 11: plants from family 0232.

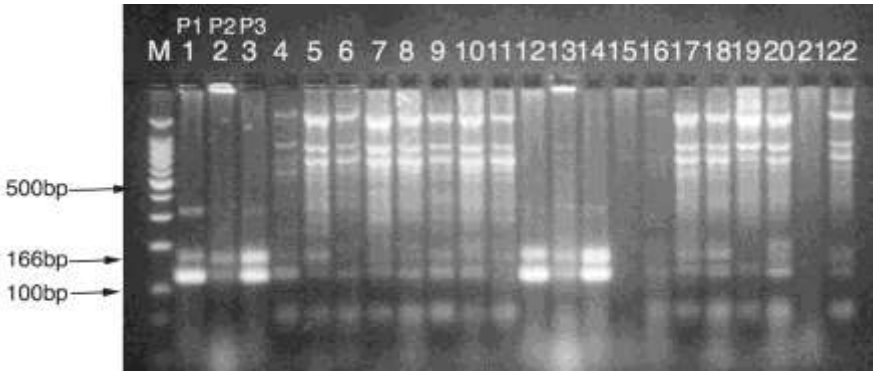


Figure 3f:-Schematic drawing of the *H22* recombinants

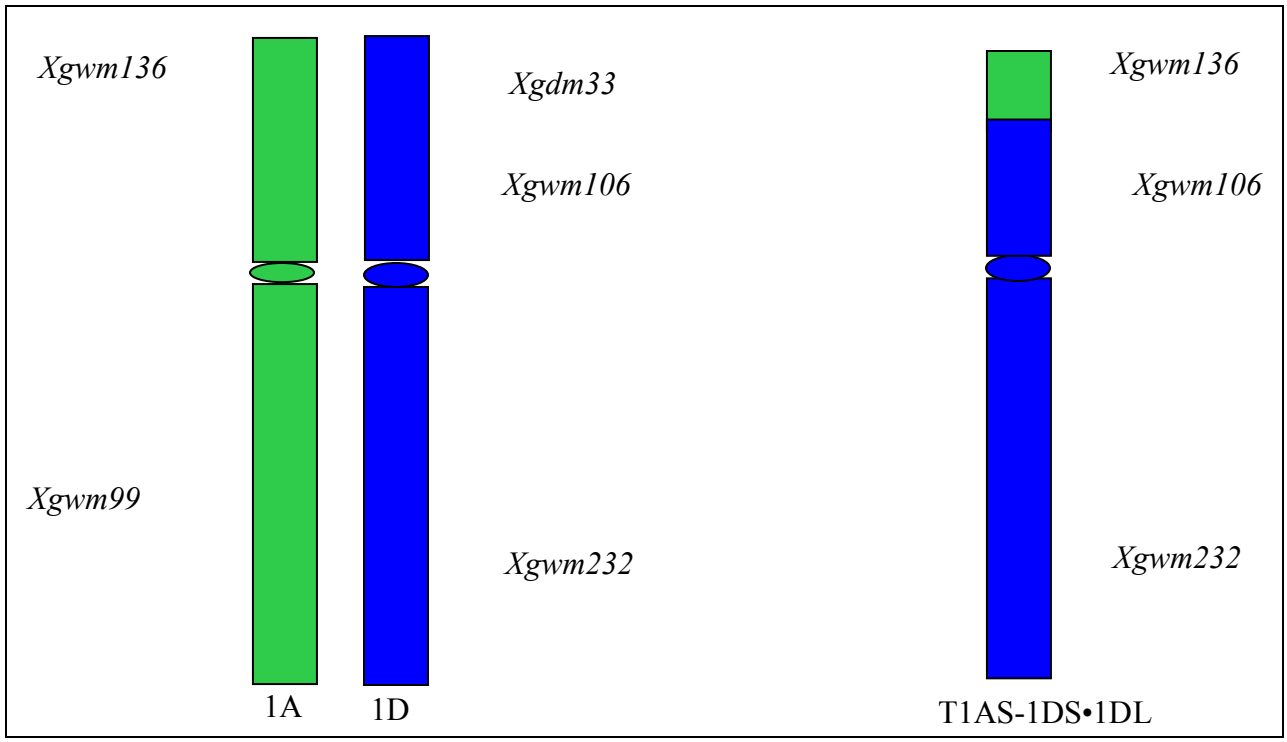


Figure 4:-Microsatellite mapping of the recombinants for the Hessian fly resistance gene *H23*.

Figure 4a:-Mapping of the microsatellite marker GWM469 (6DS, 170bp) on H23 families. Top, M: marker, lane 1: WGRC03, lane 2: LND 6D(6A), lane 3: 5D(5B), lane 4 through lane 6: plants from family 0254, lane 7: WGRC03, lane 8: LND 6D(6A), lane 9: 5D(5B), lane 10 through lane 17: plants from family 0272. Middle, M: marker, lane 1: 0257#7, lane 5 through lane 11: plants from family 0259. Bottom, M: marker, lane 1: WGRC03, lane 2: LND 6D(6A), lane 3: 5D(5B), lane 4 through lane 11: plants from family 0279.

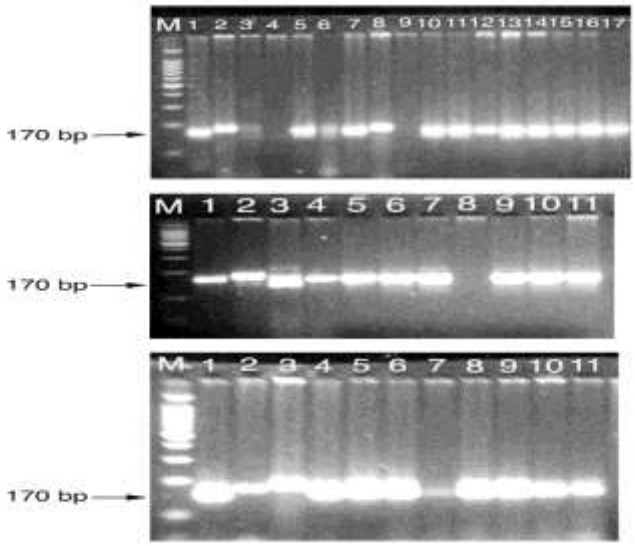


Figure 4b:-Mapping of the microsatellite marker GDM98 (6DL, 150bp) on H23 families. Top, M: marker, lane 1: LND 6D(6A), lane 2: 5D(5B), lane 3 through lane 5: plants from family 0254, lane 6: WGRC03, lane 7: LND 6D(6A), lane 8: 5D(5B), lane 9 through lane 16: plants from family 0272. Lane 17: WGRC03, lane 18: LND 6D(6A), lane 19: 5D(5B),, lane 20 through lane 27: plants from family 0279. Bottom, M: marker, lane 1 and lane 11: WGRC03, lane 2 and lane 12: LND 6D(6A), lane 3 and lane 13: 5D(5B), lane 4 through lane 10: plants from family 0257 lane 14 through lane 20: plants from family 0259.

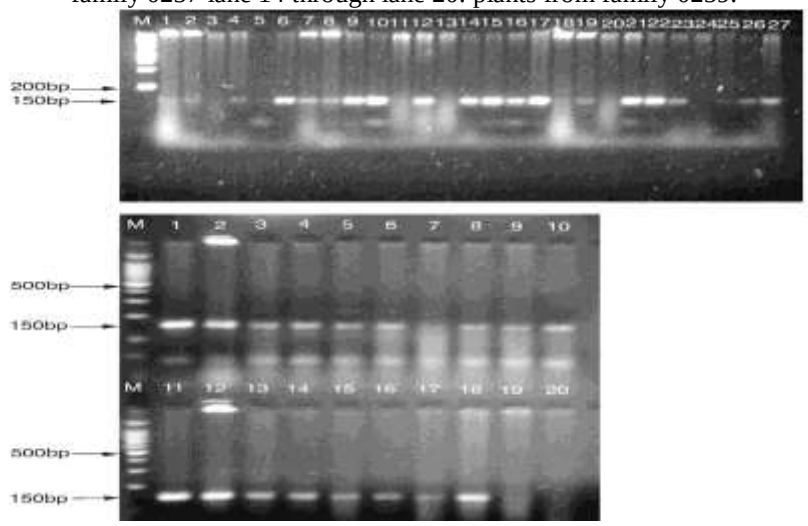


Figure 4c:-Mapping of the microsatellite markers GWM169 and GWM469 on *H23* families. Top, M: marker, lanes 1, 7 and 18: WGRC03, lanes 2, 8 and 19: LND 6D(6A), lanes 3, 9 and 20: LND 5D(B), lane 4 through lane 6: plants from family 0254, lane 10 through lane 17: plants from family 0272, lane 21 through lane 28: plants from family 0279, lane 29: gdm98 on WGRC03. Bottom: lanes 1, 11 and 21: WGRC03, lanes 2, 12 and 22: LND 6D(6A), lanes 3, 13 and 23: LND 5D(B), lane 4 through lane 10: plants from family 0257, lane 14 through lane 20: plants from family 0259, lane 24 through lane 29: plants from family 0257 amplified with gwm469.

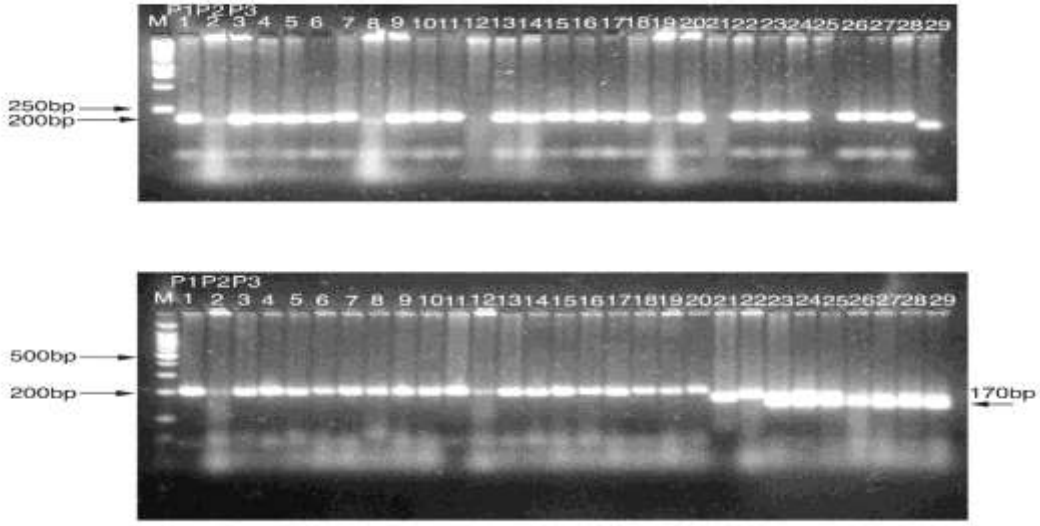


Figure 4d:-Mapping of the microsatellite marker GWM334 (6AS, 110bp) on *H23* families. Top, M: marker, lanes 1, 7 and 18: WGRC03, lanes 2, 8 and 19: LND 6D(6A), lanes 3, 9 and 20: LND 5D(B), lane 4 through lane 6: plants from family 0254, lane 10 through lane 17: plants from family 0272, lane 21 through lane 28: plants from family 0279, lane 29: gdm98 on WGRC03. Bottom, M: marker, lanes 1 and 11: WGRC03, lanes 2 and 12: LND 6D(6A), lanes 3 and 13: LND 5D(B), lane 4 through lane 10: plants from family 0257, lane 14 through lane 20: plants from family 0259.

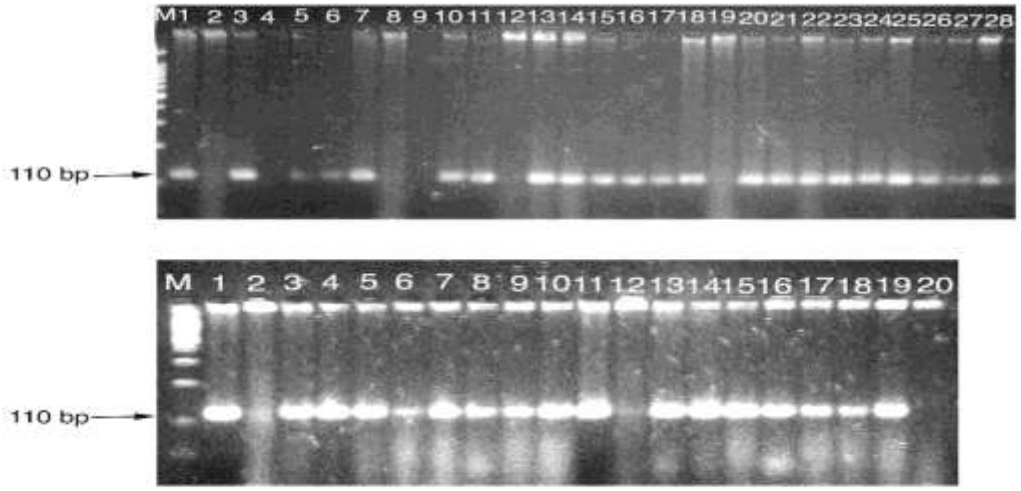


Figure 4e:-Mapping of the microsatellite markers GDM14 (6DS) and GDM132 (6DS) on *H23* families. M: marker, lanes 1 and 11: WGRC03, lanes 2 and 12: LND 6D(6A), lanes 3 and 13: LND 5D(B), lane 4 through lane 9: GDM14 on selected recombinants, lane 14 through lane 20: GDM132 on selected recombinants.

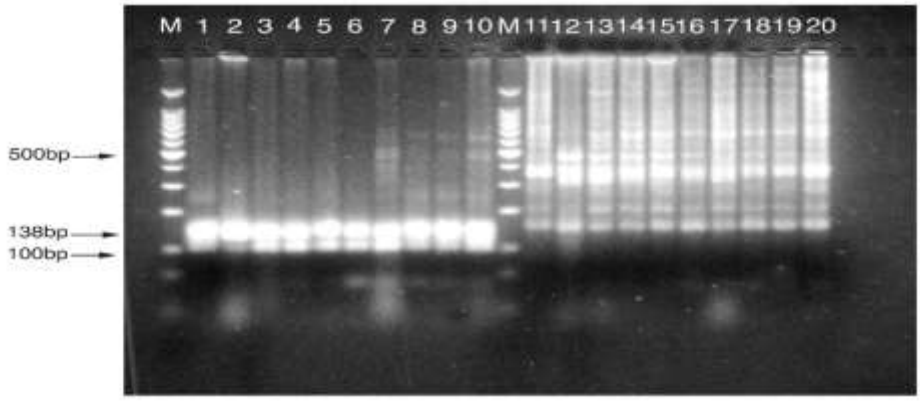


Figure 4f:-Schematic drawing of the *H23* recombinants

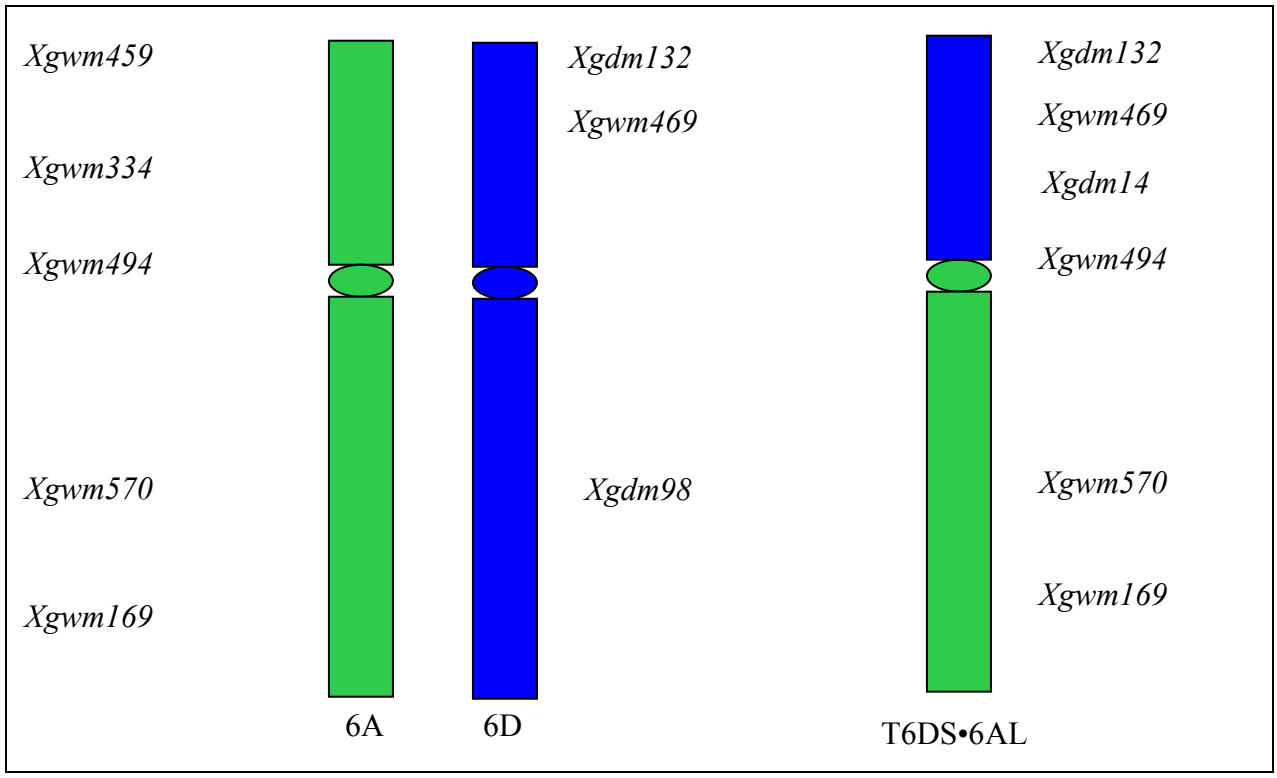


Figure 5:-Microsatellite mapping of the recombinants for the Hessian fly resistance gene *H24*.

Figure 5a:- Mapping of the microsatellites markers GWM3 and GWM314 on *H24* families. Top: GWM3 on family 0237, M: marker, Lane 1: WGRC06, Lane 2: LND 3D(3A), Lane 3: LND 5D(5B), Lanes 4 through 11: plants from family 0237. Middle: First GWM3, M: marker, Lanes 1: WGRC06, Lane 2: LND 3D(3A), Lane 3: LND 5D(5B), Lanes 4 through 9: plants from family 0241. Second, GWM314, Lanes 10 and 21: WGRC06, Lanes 11 and 22: LND 3D(3A), Lanes 12 and 23: LND 5D(5B), Lanes 13 through 20: plants from family 0237. Bottom, GWM3, Lanes 1, 10, and 19: WGRC06, Lanes 2, 11, and 20: LND 3D(3A), Lanes 3, 12, and 21: LND 5D(5B), Lanes 4 through 9: plants from family 0236, Lanes 14 through 18: plants from family 0238, Lanes 22 through 26: plants from family 0239.

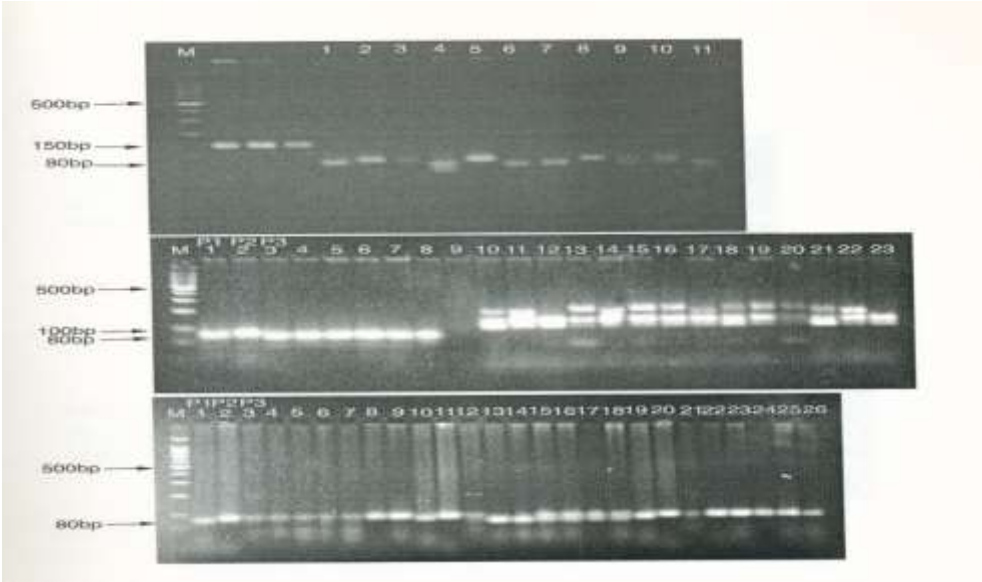


Figure 5b:-Mapping of the microsatellite markers GWM314 and GWM391 on *H24* families. Top, M: marker, Lane 1 through 6: plants from family 0241, Lane 7: WGRC06, Lane 8: LND 3D(3A), Lane 9: LND 5D(5B), Lanes 10 through 15: plants from family 0241. Bottom, M: marker, Lanes 1, 10, and 19: WGRC06, Lanes 2, 11, and 20: LND 3D(3A), Lanes 3, 12, and 21: LND 5D(5B), Lanes 4 through 9: plants from family 0236, Lanes 14 through 18: plants from family 0238, Lanes 22 through 26: plants from family 0239.

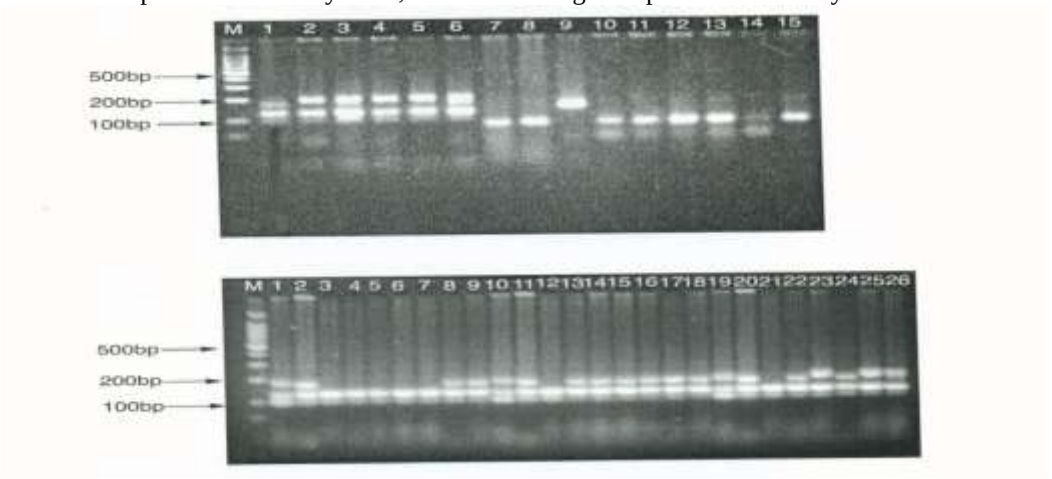


Figure 5c:-Mapping of the microsatellite marker GWM391 on *H24* families. Top: M: marker, Lane 1: WGRC06, Lane 2: LND 3D(3A), Lane 3: LND 5D(5B), Lanes 4 through 11: plants from family 0237. Bottom, M: marker, Lanes 1, 10, and 19: WGRC06, Lanes 2, 11, and 20: LND 3D(3A), Lanes 3, 12, and 21: LND 5D(5B), Lanes 4 through 9: plants from family 0236, Lanes 14 through 18: plants from family 0238, Lanes 22 through 26: plants from family 0239.

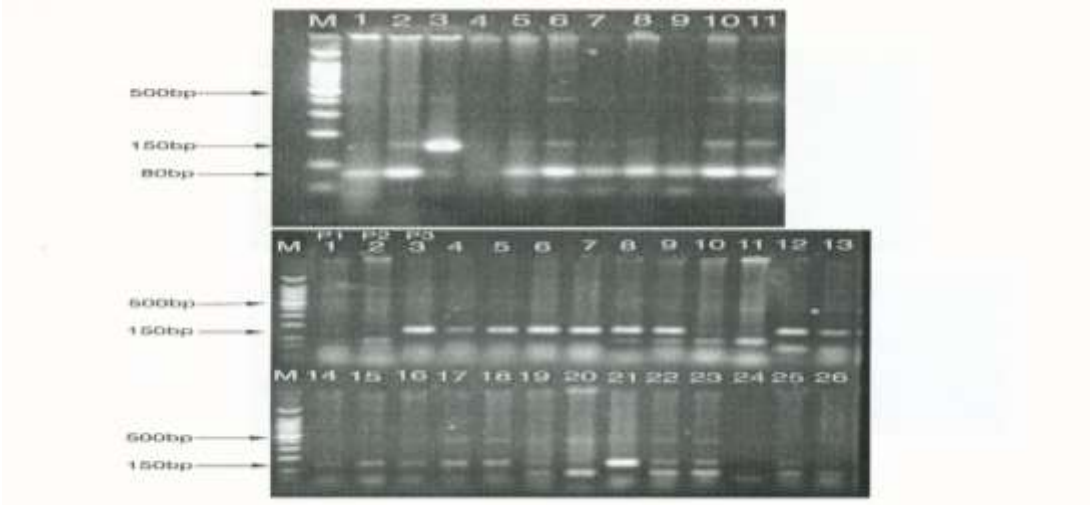


Figure 5d:- Mapping of the microsatellite marker GWM162 on *H24* families. Top, Lanes 1 and 12: WGRC06, Lanes 2 and 13: LND 3D(3A), Lanes 3 and 14: LND 5D(5B), Lanes 4 through 11: plants from family 0237, Lanes 15 through 20: plants from family 0241. Bottom, M: marker, Lanes 1, 10, and 19: WGRC06, Lanes 2, 11, and 20: LND 3D(3A), Lanes 3, 12, and 21: LND 5D(5B), Lanes 4 through 9: plants from family 0236, Lanes 14 through 18: plants from family 0238, Lanes 22 through 26: plants from family 0239.

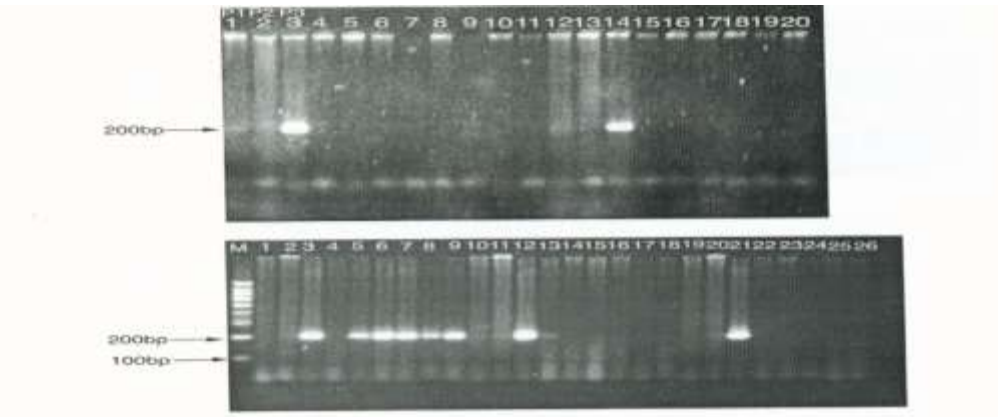


Figure 5e:-Mapping of the microsatellite marker GWM161 on *H24* families. Top, M: marker, Lanes 1 and 12: WGRC06, Lanes 2 and 13: LND 3D(3A), Lanes 3 and 14: LND 5D(5B), Lanes 4 through 11: plants from family 0237, Lanes 15 through 20: plants from family 0241. Second gwm369, Lane 21: WGRC06, Lane 22: LND 3D(3A), Lane 23: LND 5D(5B), Lanes 24 through 29: plants from family 0237. Bottom, M: marker, Lanes 1, 10, and 19: WGRC06, Lanes 2, 11, and 20: LND 3D(3A), Lanes 3, 12, and 21: LND 5D(5B), Lanes 4 through 9: plants from family 0236, Lanes 14 through 18: plants from family 0238, Lanes 22 through 26: plants from family 0239.

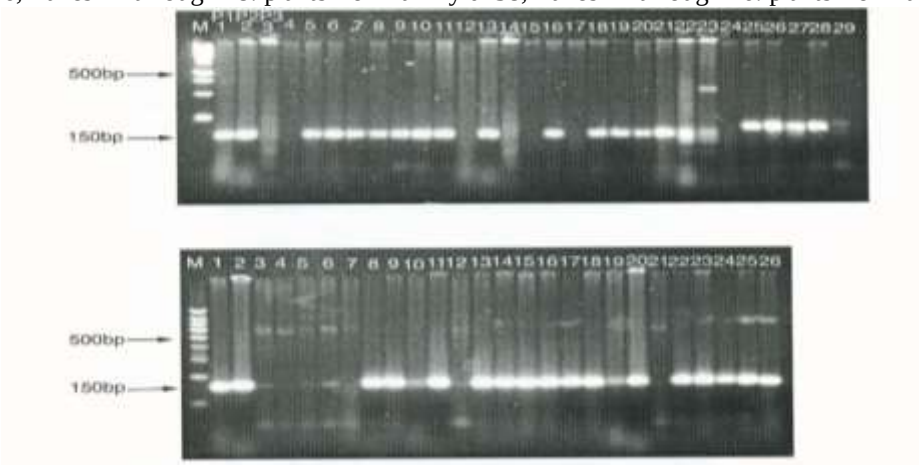


Figure 5f:-Mapping of the microsatellite marker GWM369 on *H24* families. Top, M: marker, Lanes 1 and 2: plants from family 0237, Lane 3: WGRC06, Lane 4: LND 3D(3A), Lane 5: LND 5D(5B), Lanes 6 through 11: plants from family 0241. Bottom, M: marker, Lanes 1, 10, and 19: WGRC06, Lanes 2, 11, and 20: LND 3D(3A), Lanes 3, 12, and 21: LND 5D(5B), Lanes 4 through 9: plants from family 0236, Lanes 14 through 18: plants from family 0238, Lanes 22 through 26: plants from family 0239.

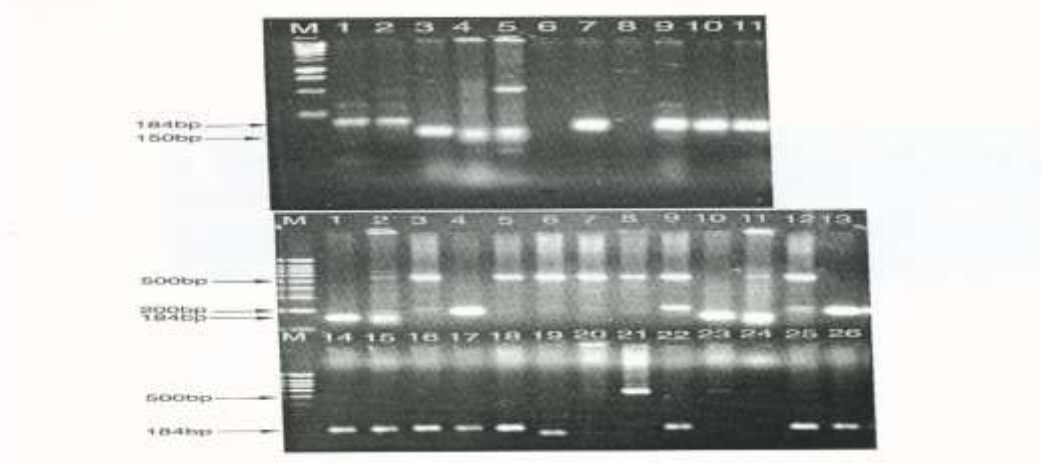


Figure 5g:-Mapping of the microsatellite marker GWM184 on *H24* families. M: marker, Lanes 1, 10, and 19: WGRC06, Lanes 2, 11, and 20: LND 3D(3A), Lanes 3, 12, and 21: LND 5D(5B), Lanes 4 through 9: plants from family 0236, Lanes 14 through 18: plants from family 0238, Lanes 22 through 26: plants from family 0239.

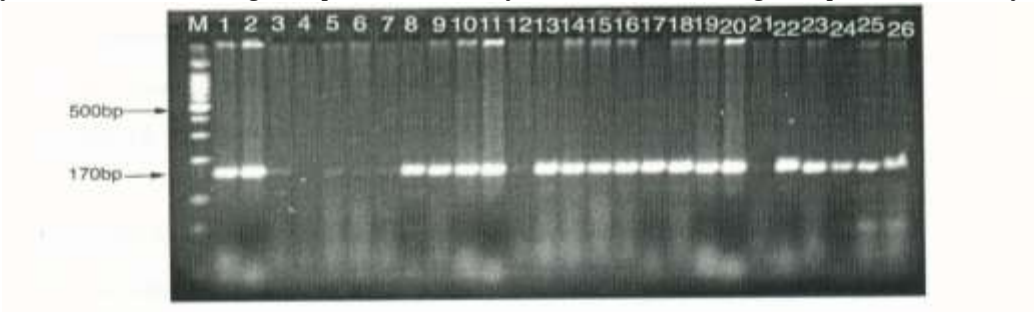
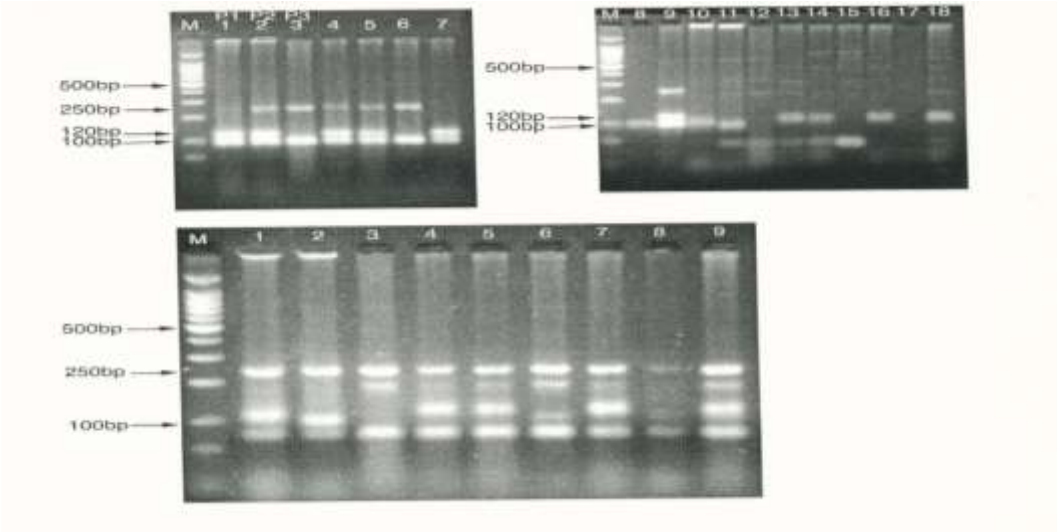


Figure 6:-Microsatellite mapping of the recombinants for the Hessian fly resistance gene *H26*.

Figure 6a:-Mapping of the microsatellites markers GWM160 and GWM601 on *H26* families. M: marker, Lanes 1 and 10: WGRC26, Lanes 2 and 11: LND 4D(4A), Lanes 3 and 12: LND 5D(5B), Lanes 4 through 9 and Lanes 13 through 18: plants from family 0244.

Figure 6b:-Mapping of the microsatellites markers GDM129 and GWM609 on *H26* families. Top, M: marker, Lanes 1 and 10: WGRC26, Lanes 2 and 11: LND 4D(4A), Lanes 3 and 12: LND 5D(5B), Lanes 4 through 9 and Lanes 13 through 18: plants from family 0244. Bottom, M: marker, Lane 1: WGRC26, Lane 2: LND 4D(4A), Lane 3: LND 5D(5B), Lanes 4 through 9: Plants from family 0244.



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