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RESEARCH ARTICLE

MOLECULAR CYTOGENETIC ANALYSIS OF RADIATION-INDUCED NEW TRANSLOCATION FROM A WHEAT LINE WITH A RYE DITELOSOME 2RL CARRYING THE HESSIAN FLY RESISTANCE GENE H21.

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Abstract

The Hessian fly resistance gene *H21* is present on the wheat-rye whole-arm translocation T2BS·2RL and was recently transferred to durum wheat. However, homozygous lines for this translocation have poor plant vigour; low seed set, and are almost completely sterile, making it impossible to use this germplasm directly in durum wheat improvement. The objective of this study was to reduce the rye segment in T2BS·2RL using *irradiation*, thereby making this gene available for wheat breeding. The plant material used in this study consisted of the *H21* durum ditelos lines for chromosome 2RL. The mature pollen was collected from grown ditelos 2RL lines and was irradiated using the X-rays and then used to pollinate normal durum wheat plants. The Hessian fly resistant lines were analyzed using C-banding and GISH techniques. These techniques have revealed two newly recovered translocations. The resistant lines have retained distal part of rye chromosome 2R that carries the resistance gene *H21* in the form T2AS.2AL-2RL translocation. Such recombination events are not of agronomic interest because they retain a nearly complete 2RL arm that has deleterious effects. Whereas, the susceptible lines had a small segment of proximal rye chromosome 2RL in the form of T2RL-2AL. This part of rye chromosome does not carry the resistance gene. This study has confirmed the utility of using the irradiation technique to reduce chromosome arms of the wild relatives such rye. Also, we have confirmed that irradiation is still random in breaking chromosomes and several translocations could be generated but the cytogenetic techniques such as C-banding and genomic in situ hybridization are very powerful techniques for identifying and selecting more interesting translocations.

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Introduction:-

The Hessian fly, *Mayetiola destructor* (Say), is a major insect pest of wheat. Genetic resistance is the most effective and economical means of controlling this insect (Ratcliffe and Hatchett 1997). To date, 31 Hessian fly-resistance genes (H1 through H31) have been identified in *Triticum/Aegilops* species and in *Secale cereale* L. (McIntosh et al., 1998). The mechanism of resistance conditioned by these genes is antibiosis, whereby 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.

Lacking adequate levels of resistance, wheat is usually damaged severely by Hessian fly in the dry areas of northern Africa. In Morocco, yield losses due to damage by this insect have been estimated at up to 36% of the yearly small grain production (Lhaloui et al. 1998). Among the identified genes for resistance to Hessian fly, ten have been found to be effective against the Moroccan populations of the insect and have been widely used in cultivar development of bread wheat (El Bouhssini et al. 1998). However, only one source of resistance was identified in durum wheat.

Cultivated rye (*Secale cereale* L.) is an important source of genes for insect and disease resistance in wheat. To date, 12 genes conferring resistance to various diseases and insects have been transferred from rye into wheat (Friebe et al., 1996). The first report of gene transfer from rye was the transfer of greenbug resistance from rye to wheat by an apparent radiation-produced chromosomal translocation (Sebesta and Wood, 1978). Sears (1956) was first to use ionizing radiation as a method for recombining alien genetic material with that of wheat, he transferred a gene for leaf rust resistance from *Aegilops umbellulata* to hexaploid wheat. Since then, irradiation has been used widely for transferring desirable alien genes to wheat. Recently, Hessian fly (*Mayetiola destructor* (Say)) resistance genes H21 and H25 were transferred from rye chromosome arms 2RL and 6RL to wheat via homoeologous recombination T2BS.2RL and via radiation-induced terminal T6BS.6BL-6RL, T4BS.4BL-6RL and intercalary Ti4AS.4AL-6RL-4AL wheat-rye chromosome translocations (Friebe et al 1990, 1991;). They were first transferred to bread wheat (*Triticum aestivum* L.) in the form of a Robertsonian wheat-rye T2BS·2RL translocation using tissue culture (Friebe et al. 1990). Then transferred to durum wheat (Friebe et al. 1999). Homozygous H21 durum plants have poor plant vigor and are almost completely sterile. By using the homoeologous recombination, Ferrahi et al. 2017a were able to reduce the rye chromatin the initial translocation T2BS.2RL.

Genomic in situ hybridization (GISH) using total genomic DNA as probe was shown to be highly sensitive technique. It allow in detecting alien chromatin in wheat thus permitting the analysis of chromosome and genome arrangements within a nucleus and allowing the determination of the translocation breakpoints (Le et al. 1989, Mukai et al. 1993; Friebe et al., 1992; Jiang et al. 1994a).

In this work, we report the characterization new translocation obtained after the pollen irradiation to reduce the 2RL rye chromosome long arm using the ditelos lines 2RL (Figure 2A) and normal for chromosome 2A. These lines were analyzed using the GISH technique.

Material and Methods:-

Plant Material and GISH:-

The plant material used in this study consisted of the H21 durum ditelos lines for chromosome 2RL obtained from Dr JH Hatchet at Kansas State University. All lines are maintained at the Wheat Genetics Resource Center at Kansas State University, Manhattan. Plants 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. Chromosome identification during the course of line selection was according to the standard C-banding protocol described by Gill et al. (1991) and Genomic In Situ Hybridization (GISH) was according to Jiang et al. (1994a).

Irradiation Protocol:-

The mature pollen was collected from grown ditelos 2RL lines and was irradiated using the X-rays machine. The optimal dose was selected after several combination of time duration and dose using the check. The selected dose that has given good results was 4 minutes and 120Kvp.

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. Biotype L is the most virulent biotype presently found in the field. Greenhouse temperature was maintained between 18°C and 24°C 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. Susceptible and resistant plant reactions were 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:-

GISH and C-banding analysis:-

Root tips from several irradiated ditelos 2R plants were collected and analyzed by GISH (Figure 2a). The results of Genomic in situ hybridization showed that we have generated a new translocation where only a few rye chromatin is sitting near the centromere of the ditelos and the a big segment of rye chromatin is translocated to wheat chromosome 2A long arm. Further analysis using C-banding has confirmed that the new generated translocations are T2AS.2AL-2RL and T2RL-2AL (Figures 1a, 1b, 1c and 1d). These latter translocations can be drawn as shown in Figure 2b. The breakage and chromosome fusion after the effect of irradiation has occurred between chromosome arm 1RL and chromosome arm 1AL. Several previous studies have reported that the long arms of rye chromosomes 1R and 2R pair relatively frequently with their wheat homoeologous (Naranjo and Fernandez-Rueda 1996; Lukaszewski 1993, 1997 and 2000).

Hessian fly testing :-

The Hessian fly testing of the recovered translocations showed that lines in Figures 1a, 1b and 1c were resistant to the insect because they retained distal part of rye chromosome 2R that carries the resistance gene H21 in the form T2AS.2AL-2RL translocation. Such recombination events are not of agronomic interest because they retain a nearly complete 2RL arm that has deleterious effects. Whereas, the lines in figure 1d were susceptible as they had a small segment of proximal rye chromosome 2RL. This part of the chromosome does not carry the resistance gene H21.

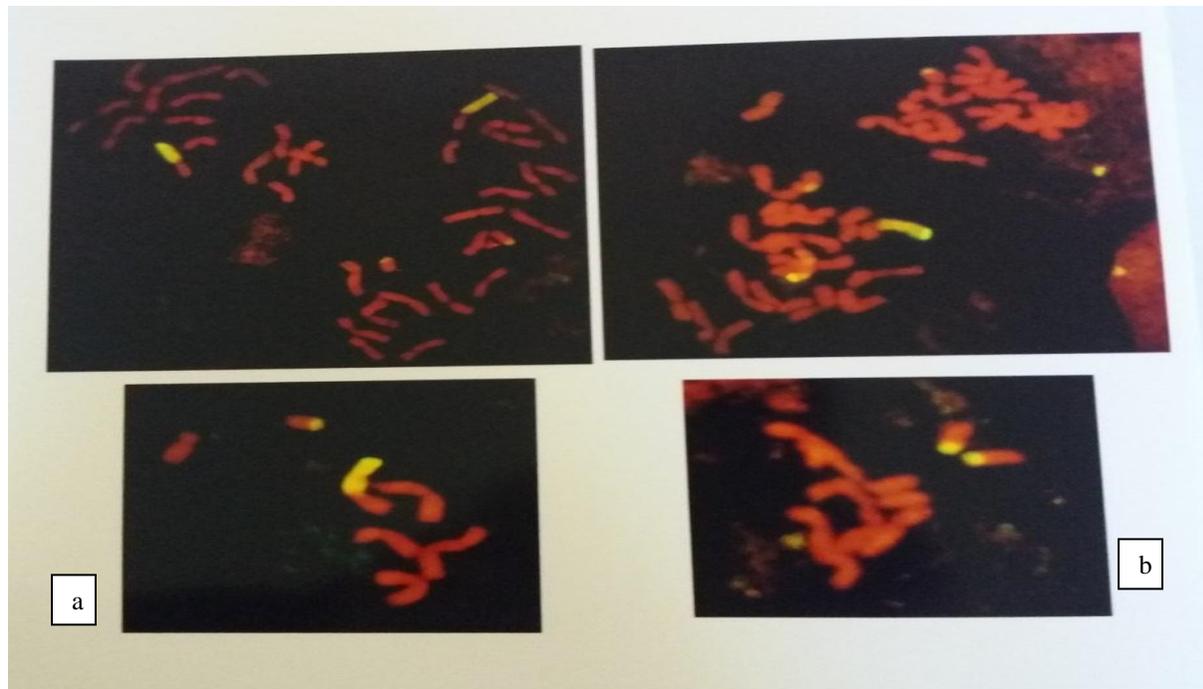


Figure 1:-GISH pattern of recovered primary new translocation after irradiation. Rye chromatin is visualized by yellow FITC fluorescence, whereas wheat chromatin is counterstained with PI and fluoresces red. (a) Meiotic metaphase I T2AS.2AL-2RL and T2RL-2AL, this later translocation is in the form of ditelos; (b) close metaphase I

of the new translocation T2AS.2AL-2RL; (c) very close picture showing metaphase I of one chromosome of the new translocation T2AS.2AL-2RL and one ditelos; (d) very close picture showing metaphase I of the two ditelos.

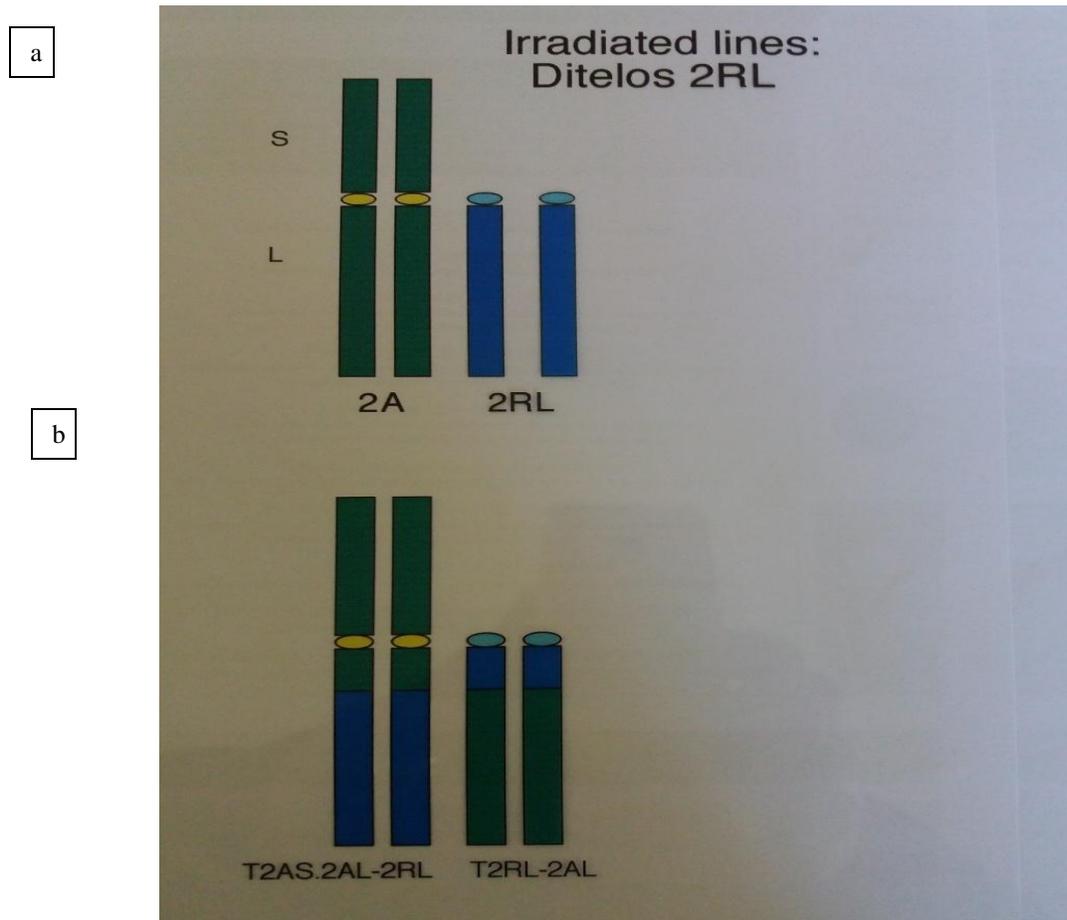


Figure 2:-Drawing on the irradiated lines (a) and the new generated translocation (b) after the irradiation of the ditelos 2RL plants

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 contributed in cultivar development because of the non-compensating nature of transfers (Friebe et al., 1996).

Successful gene transfers from alien species rely on the ability of the chromosomes to recombine with homoeologous wheat chromosomes. In wheat, chromosome pairing is restricted to homologous chromosomes, which is controlled by the Ph1 locus on chromosome arm 5BL (Okamoto 1975; Riley and Chapman 1958; Feldman 1993). Two deletions in this locus have been identified, one in hexaploid wheat (ph1b, Sears 1977) and the other in tetraploid wheat (ph1c, Giorgi 1978). Riley and co-workers (1968a, b) were the first to use induced homoeologous recombination to transfer genes conferring resistance to stripe rust (Yr8) and stem rust (Sr34) from *Aegilops comosa* Sibth. And Smith ($2n=2x=14$, MM) to wheat.

Although a considerable number of successful alien introgressions involving homoeologous, chromosome segments have been achieved in hexaploid wheat (for review see Sears 1993; Friebe et al. 1996; Lukaszewski 2000). Very little progress has been made in durum wheat (Dvorak and Lukaszewski 2000; Dvorak and Gorham 1992; Dvorak et al. 1994; Luo et al. 1996b; Ceoloni et al. 1996, Ferrahi et al., 2017a, Ferrahi et al., 2017b), probably because the

buffering ability of tetraploid durum wheat is less than that of hexaploid wheat. Other sources of resistance need to be used in durum wheat improvement to increase the genetic variability. Ferrahi et al., (2017a et 2017b) have reported the transfer of Hessian fly resistance genes derived from *Aegilops tauschii* (H22, H23, H24, and H26) and two Hessian fly resistance derived from rye (H21 and H25).

As shown by C-banding the new translocation has occurred between the chromosome arm 1RL and chromosome arm 1AL. The breakage and chromosome fusion after the effect of irradiation has occurred between chromosome arm 1RL and chromosome arm 1AL. Several previous studies have reported that the long arms of rye chromosomes 1R and 2R pair relatively frequently with their wheat homoeologous (Jauhar et al., 1991 and 1999; Naranjo and Fernandez-Rueda 1996; Martinez-Perez et al. 1999; Lukaszewski 1993, 1997 and 2000).

The first step is the production of genetically compensating translocations and several of such compensating wheat-rye translocations are available (Friebe et al., 1996). The second step of such transfers can be accomplished either by homologous recombination to stack more genes in the alien chromosome arm as was first done (Friebe et al. 1994; Jiang et al., 1994b) in transferring the powdery mildew gene Pm20 from 6RL to the existing translocation T6BS·6RL. In the case of non-homologous arms, the reduction of the alien segment can be accomplished by induced homoeologous recombination as was shown by Ferrahi et al. (2017a).

In this study, we were able to rearrange the initial ditelos in a new translocation involving chromosome 2A of wheat. This generated new translocation has given Hessian fly resistant lines but still contain more rye chromatin that could have deleterious effects. Further work is needed to reduce the rye arm in the translocation using homoeologous recombination.

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