Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
an open access publisher
http://ansinet.com

Plant Pathology Journal 5 (1): 41-46, 2006
ISSN 1812-5387

Two Adapted Techniques in Studies of DNA Fingerprinting of *Septoria tritici* Populations

[1]C.A. Cordo, [2]M. de las M. Lojo and [3]P. Remorini
[1]Comisión de Investigaciones Científicas, Buenos Aires Province and CIDEFI-Facultad de Ciencias Agrarias y Forestales de La Plata, UNLP, Calle 60 y 119, (1900) La Plata, Pcia, de Buenos Aires, Argentina
[2]Department of Microbial Genetic, Facultad de Veterinaria, UNLP,
Calle 60 y 118, (1900) La Plata, Pcia, de Buenos Aires
[3]CONICET at the CEVAN, Centro de Virología Animal,
Serrano 669, (1427), Cap. Fed., Argentina

Abstract: In this study two new protocols with nonradioactive techniques applied on DNA analysis with RFLP (Restriction Fragment Length Polymorphism) were presented. They have been modified from the DIG (Boehringer Mannheim) and Photogene 2.0 (Life Technology) Systems. DNA probes were random-primed labeled with the specific compounds for each system. The quantity of digested DNA, the concentrations of the probe in the hybridization step and the concentration of the anti-body conjugate for the detection process were optimized. The improvements from the original protocol for the DIG System were an increase in the concentration of the labeled probe in the hybridization step and in the concentration of the anti-digoxigenin-AP conjugate in the detection process. There were no quantitative changes in the protocol of the Photogene System, but the quality of the washing process was improved for the post hybridization, blocking and detection steps. There is an economic advantage when using the Photogene System if the cost for reaction is analyzed. A brief comment about the frequency of the RFLP patterns from the isolates of both geographical sampled regions was also presented. The following advantages were found when these systems were used: safety in handling, no decay of labeled probes, reusable hybridization mixes (in the case of the DIG System), extremely short exposure times in the signal detection and economical differences in the cost per reaction for the Latin American economical conditions. This procedure saved time and cut costs without reducing sensitivity.

Key words: DNA hybridization, nonradioactive technique, *Septoria tritici-Mycosphaerella graminicola,* RFLP

INTRODUCTION

Septori tritici Rob ex Desm. is a foliar pathogen of wheat and several other grasses[1,2]. The conidia are dispersed by rainsplash, therefore having limited potential to move over short distances, while the ascospores are wind-dispersed, hence having the potential to move over longer distances. This mode of dispersion allows to study the fungus population dynamics through genetic analysis. The primary genetic marker used for *Septori tritici* was virulence, studied on a set of wheat differentials that varied in their level of resistance. However, virulence in this fungus is a character that is very sensitive to environmental conditions, originating difficulties to evaluate it in a reproducible manner.

McDonald and Martínez[3,4], developed a set of genetic markers based on RFLP that could be used to estimate the amount and distribution of genetic variability in this fungus. All of them are based on DNA radioactive labeling. Genetic variation was assessed using individual RFLP loci to measure gene diversity and DNA fingerprints to identify clones and measure genotypic diversity[5-9].

In Argentina, the physiological specialization of this fungus has been widely studied[10-15]. All the results have confirmed that this fungus shows variation in the degree of virulence. However, the genetic variation among the populations in Argentina is unknown. As Argentinian and some other laboratories in the world are not always equipped to work with radioactivity. Two nonradioactive techniques have been developed to work on the genetic characterization of *S. tritici* isolates.

The goal of this study was to describe, in a comparative way, the improved protocols of both nonradioactive techniques (DNA DIG System and Photogene version 2.0) and recommend their use for economic and safety reasons.

Corresponding Author: Cristina A. Cordo, Centro de Investigaciones de Fitopatología, CIDEFI, Phytopatology Lab, Facultad de Ciencias Agrarias y Forestales, UNLP, C.C. 31, 1900 La Plata, Pcia. de Buenos Aires, Argentina Tel: (054) 221-423-6758 Fax: (054) 221-425-2346

MATERIALS AND METHODS

Field sampling and sample preparation: The leaf infected tissues were collected from plants on GS10.1 stage[16] in Los Hornos and Balcarce localities (Buenos Aires Province, Argentina). Hierarchical sampling methods were used with each plant to collect *S. tritici* isolates from a naturally infected wheat field. Thirty eight isolates originated from 38 lesions on 13 leaves were sampled at three Los Hornos sites in a single field. Each of them was approximately 10 m^2 in area. Ninety eight isolates originated from 98 lesions on 37 leaves were sampled at 8 balcarce localities separated 10 m from each other. The total sampled area was 80 m^2. The infected wheat leaves were air-dried at room temperature for 2 weeks before the fungi isolations were done. Only one single-spore isolate was obtained with the disinfection technique (alcohol 70% and Cl_2 Hg 1/1000 g mL^{-1}) and cultivated on PDA (2%)[17]. The isolates were grown in yeast sucrose broth[4] at room temperature (18-22°C) during 10 days with shaking (100-150 rpm) to obtain the DNA.

DNA extraction and probe: DNA was extracted from each isolate by a CTAB extraction protocol described previously[3]. Two grams of mycelium yielded 2.0-5.0 ug μL^{-1} of DNA. The purified DNA was digested by following a protocol that combined the digestion and posterior precipitation of already digested DNA. All the preparations were done on ice The DNA preparation (50 ng μL^{-1}) were digested with Pst1 enzyme through the following Master mix: tri-distilled water, 153 mL; buffer H (Promega), 40 μL; BSA, 4 μL; Pst1 enzyme (Promega 10 units μL^{-1}), 3 uL. For each isolate, mixed in an Eppendorf tube: 60 uL of DNA, 140 uL of tri-distilled water and 200 uL of the enzyme mix. After mixing, incubate 2 h at 37°C. Spin at 16000rpm 10 min and take the supernatant. Add 200 uL 7.5 M LiCl, vortex 5 sec. Put on ice 10 min. Spin at 16000 rpm, 10 min. Transfer the supernatant to a fresh 1.5 mL tube and add 1 mL of 95% v/v EtOH at room temperature. Vortex 5 sec. Let tube sit at room temperature for 20-30 min. Spin down at top speed in microfuge for 15 min. Pour off the EtOH and allow all the EtOH to volatilize off and then suspend the pellet in 40 uL of distilled water. Place tube in a 65°C water-bath for 5 min; vortex 10 sec; again 65°C for 5 min; vortex 10 sec; spin down. Keep in a fridge before the control of DNA concentration.

Development of anonymous pSTL 70 DNA repetitive probe was described previously[4]. The plasmid was recuperated from the *Escherichia coli* (HB101 strain) culture with the wizard Maxiprep DNA Purification System (Promega).

The sensitivity of the detection process depended on the probe that was labeled by a random-primed method with different compounds following the manufacture's recommendations.

DIG DNA labeling and detection system (Boehringer mannheim): DIG-11-dUTP was used to label the DIG DNA[18,19]. The sensitivity of DIG detection of the former depended on the concentration of the DIG labeled probe in the hybridization reaction and on the duration of the colour reaction.

Electrophoresis, blotting and hybridization were performing as follow: Digested DNA (3 ug/lane) was separated in 1.0% agarose TBE gel for 16-18 h at 2.4 V cm^{-1} and then transferred onto a nylon membrane (Hybond ™–N, Amersham, International, Buckingamshire, UK), by the alkaline transfer method[20]. Blotter transfer (Vacuum Blotter Appligene) was conducted during 1h at 45-50 mb of pressure with 10X SSC solution. Prehybridization and hybridization reactions were performed according to the manufacture's instructions in a Standard hybridization oven (Appligene) at 65°C[3,4]. The hybridization solution was performed with 2.5 mL of hybridization solution per 100 cm^2 of membrane and 26 ng of the probe per mL of the same solution.

Photogene 2.0 system (Life technology): Biotin-14-dCTP was used to label the probe in the Bio Prime System[21]. Digested DNA (3 ug/lane) was separated in 1.0% agarose TBE gel for 16-18 h at 2.4 V cm^{-1} and then transferred onto a nylon membrane Pall Biodyne A, according to the system, by the alkaline transfer method[20]. Blotter transfer (Vacuum Blotter Appligene) was conducted during 1h at 45-50 mb of pressure with 10X SSC solution. Prehybridization and hybridization reactions were performed according to the manufacture's instructions in a standard hybridization oven (Appligene) at 65°C[3,4].

The hybridization solution was performed with: 0.1 mL of hybridization solution per 100 cm^2 of membrane and 50 ng of the probe per mL of the same solution.

Washing process: Washing, after hybridization, with the DIG System, was carried out in tubes in the hybridization oven following the McDonald and Martínez protocol[4].

The manufacture's instructions for the Photogene System were strongly modified on frequencies and times: the first wash was carried out at hybridization temperature (65°C), three times, 5 min per wash, instead of twice as was indicated on the original instruction; the second wash was carried out at 50°C, three times, 20 min per wash, instead of once; the third and fourth washes were done out of the oven, in a plastic tray, shaking one or two

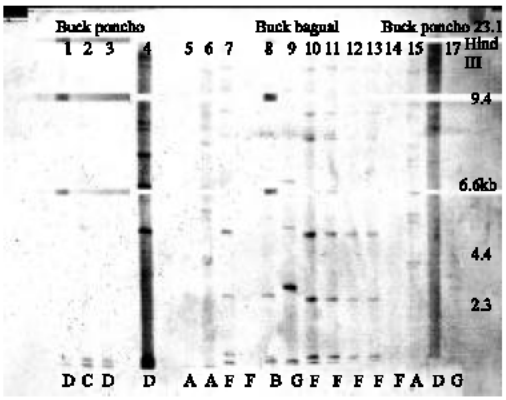

Fig. 1: Labeling and detection of nonradioactive hybridization with DIG system. Restriction Fragment Length Polymorphism (RFLP) fingerprint patterns (pSTL70) on the genotypes of *Septoria tritici* isolates from two wheat cultivars (Buck Poncho and Buck Bagual) of Los Hornos, province of Buenos Aires. Probe hybridized the DNA digested by Pst 1. The first lane at the left is the Hind III size standard marker; capital letters represent different patterns of the present genotypes. Code of isolates: from Buck Poncho origin, 1-PE11, 2-PE32, 3-PC12, 4-PE13, 5-PC13, 6-PD23, 7-PD22, 15-PD12, 16-PE12, 17-PF11; from Buck Bagual origen: 8-BBLH11, 9-BBLH13, 10-BBLH21, 11-BBLH23, 12-BBLH31, 13-BBLH32, 14-BBLH33. -isolates 4, 5 and 6 were collected from lesions of different plants from Buck Poncho cultivar. -isolates 6 and 7 were collected from different lesions of the same plant. -isolates 8, 9 and 10 were collected from lesions of different plants from Buck Bagual cultivar. -isolates 10 and 11 were found within different lesions of the same plant from the same cultivar

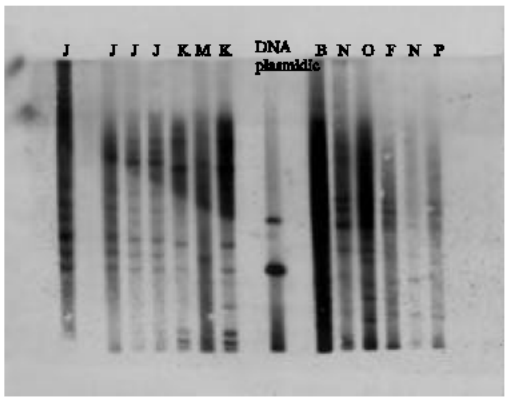

Fig. 2: Labeling and detection of nonradioactive hybridization with the Photogene 2.0 system. DNA fingerprints of *S. tritici* isolates from Balcarce, province of Buenos Aires. pSTL 70 probe has hybridized the Pst 1 digested DNA. Each lane with its letter is related to different types of patterns. Three ul of plasmidic DNA (50 ng μL^{-1}) were loaded as a sensitivity control

times, 5 min per wash. The following steps were done without protocol modifications.

Detection process: The sensitivity in the detection of the labeled DNA was improved under different ways depending on the nonradioactive technique. In the case of the DIG System[19], DIG –labeled hybrids were detected with an anti-DIG- alkaline phosphatase conjugate and the NBT (nitroblue tetrazolium salt) and BCIP (5-bromo-4-chloro-3-indolyl phosphate, toluidine salt) substrates, which gave a light-blue precipitate. The improvement on this step was to modify the concentration of the Anti-digoxigenin-AP-conjugate in the washing buffer (40 µL of the conjugate in 20 mL of buffer 1; it represents a change in the concentration at 1:500 v/v instead of 1:1000 v/v, as the protocol is indicating).

The detection process involved two basic steps for the Photogene System: 1) streptavidin-alkaline phosphatase (SA-AP) conjugate was bonded to the biotine groups of the labeled probe; 2) the membrane was incubated with alkaline phosphatase substrate that emits light when dephosphorylated. This signal is detected by exposure to X-ray film used for autoradiograph. The first change in these processes was in binding the streptavidin-alkaline phosphatase conjugate step: we placed the hybridized membrane into the hybridization tube instead of placing it into a hybridization bag or plastic container and we added the prewarmed (65°C) blocking solution. After 1 h at that temperature, the membrane may be washed five or six times at room temperature with TBS-Tween 1:10 of the total volume of the tube for 30 sec per wash. The final steps were performed following the protocol without modifications.

Signal detection: The time of the signal detection varied for each system. When using the DIG System, the signal detection appeared at 10-60 min of exposure to the colorimetric reagent, at room temperature and only one hybridized membrane could be obtained (Fig. 1). On the other hand, when using the Photogene System, the detection reagent (Lumi-phos 530) produced a chemiluminiscent emission that was visible between 5 and 15 min after x-ray film (Kodak XAR or equivalent) exposure (Fig. 2). The advantage of this constant emission (relatively constant, at room temperature, during 24 h) is that it allows multiple exposures with good resolution from a single blot.

RESULTS AND DISCUSSION

The nonradioactive systems are able to fulfil all criteria, providing the same level of sensitivity and easy handling as radioactive methods but avoiding all hazards and inconveniences. Both populations showed different numbers of polymorphic patterns with a variable number of fragments when the digested DNA of the different isolates were hybridized by pSTL 70 probe (Table 1).

In this study, the use of sequences of repetitive labeled DNA with nonradioactive compounds was useful as a probe for DNA fingerprinting. It was labeled with digoxigenin or biotine nucleotides in all the isolates from the two wheat localities (Los Hornos and Balcarce). From 136 isolates coming from different areas of the Argentine wheat region, 20 isolates from Los Hornos were characterized with the DIG System and 31 isolates from Balcarce were with the Photogene version 2.0. The pSTL70 fingerprinting probe hybridized many DNA fragments of different sizes in isolates from field populations of both places. All leaf samples went through the same process of isolation, culture, DNA extraction, alkaline blotting and probing. Some of the isolates did not yield good quality DNA for the restriction enzyme digestion process. This explains the loss of isolates in the samples of the populations.

The average number of hybridized fragments between 0.5 and 9.4 kb was from 4 to 11 and their average weight ranged from 2.2 to 8.89 kb. A total number of 16 polymorphic patterns were developed on 51 isolates (Table 1). Six of them were repeated in both populations (A with 15 and 3.22%; B with 10 and 6.45%; C with 5 and 9.70%; D with 20 and 19.35%; F with 20 and 12.90% and G with 15 and 3.22% of appearance frequency) for Los Hornos and Balcarce, respectively. Moreover, other patterns appeared with different frequencies in the last mentioned population.

In previous (unpublished) studies and at a macrogeographical level analysis, Los Hornos and Balcarce populations had similar genetic structure. The mean genetic diversity between populations-Nei's formula- was 0.31 for Balcarce and 0.26 for Los Hornos for the 8 RFLP loci. However, the result of the analysis at a microgeographical level (leaves from different wheat varieties) for Los Hornos population (Fig. 1), demonstrated three different genotypes for the Buck Poncho host variety, from a total of three leaves of different plants. They are isolates of the population that were collected among plants of this cultivar (isolates 4, 5 and 6) and among different lesions on the same leaf (isolates 6 and 7) for the same plant. Another 3 different genotypes (isolates 12, 13 and 14) were identified for the Buck Bagual variety comparing different lesions in the same leaf and among different leaves from the same plant (isolates 9, 10 and 12). The microgeographical level analysis showed a higher variation on type and number of genotypes for the Balcarce population than for the Los Hornos population (Table 1). General, different genotypes were often found within a single lesion and most lesions on the same leaf had also different genotypes. This result demonstrated, in coincidence with Boerger *et al.*[5] that a lesion is often the result of a coinfection by two or more genotypes. Most of the isolates in the Los Hornos samples with the same multilocus haplotype were isolated from the same leaf. In the Balcarce field, each multilocus haplotype is repeated one or two times as a maximum; but in Los Hornos one of them is repeated twenty one times in coincidence with its fingerprints pattern. These haplotypes are considered clones (manuscript in preparation). They appeared because Los Hornos are not an endemic area for the leaf blotch of wheat and the varieties that are checked for resistance are artificially inoculated with the pathogen, then the gene diversity is low in this field. In coincidence with McDonald *et al.*[8], these isolates could be individuals that were not sexually compatible to create mayor diversity.

Table 1: Common hybridization patterns of *S. tritici* isolates from two wheat localities

Type of pattern	Number of fragments	Frequency of patterns (%) in Los Hornos population	Frequency of patterns (%) in Balcarce population
A	6	15	3.22
B	6	10	6.45
C	7	5	9.70
D	6	20	19.35
E	6	20	3.22
F	7	15	12.90
G	7	-	3.22
H	9	-	6.45
I	4	-	3.22
J	9	-	6.45
K	8	-	3.22
L	9	-	3.22
M	11	-	3.22
N	11	-	6.45
O	10	-	3.22
P	9	-	3.22

The amount of gene flow among populations was high when all the populations were compared. The Nm mean value (3.1563) indicated that 315 individuals were moving every year between localities. As McDermott and McDonald thought[22] it created a substantial differentiation between those populations. Also here, the movement of individuals among populations was observed using the DNA fingerprinting method. It was found that some of the isolates with the same fingerprinting hybridization pattern also had the same alleles at individual RFLP loci (unpublished). Then, they were considered clones that shared both places at the same time. The same individual has been found in two

close populations because asexual reproduction, specially in Los Hornos, is a significant component of the pathogen life cycle in Argentina and its descendant would be widely dispersed over distance. In this particular case, it could be understood that the genetic similarity between two geographic areas distant 400 km from each other, as in this case, would depend largely on the dispersal ability of the organism. Cordo *et al.*[23] determined that pycnidiospores were produced in greater abundance than ascospores and they were the predominance sources of inoculum in the Los Hornos field. Moreover, in the Balcarce field, throughout the gene diversity results, the most obvious dispersion method apparently be air dispersal of ascospores[9,22] that have the potential to move at least several tens of kilometers; may be that exist an alternate host of *S. tritici*, producing pycnidia, that constitute a continuous host population where ascospores could move readily among local geographic populations[5]. They maintain a uniform source of inoculum that infects the wheat field each autumn.

Both non-radioactive techniques offer several advantages over previous radioactive methods for the detection of RFLP fragments: Is safety on handling, no hazards to health or the environment, no inconvenient safety restrictions.

For DIG System, no decay of labeled probes: DIG- labeled probes are stable for more than one year; Reusable hybridization mixes: only DIG –labeled probes can be stored in the hybridization buffer at 20°C and can be reused several times when freshly denatured. The total concentration of the probe in the hybridization solution was duplicated to 2.6 μl mL^{-1} of it. Moreover, the concentration of the anti-DIG- antibody conjugated reagent into buffer1 solution was also duplicated (1:500 v/v instead of 1:1000 v/v).

For (Photogene system) Biotine-label probes are only stable for 6 months. Extremely short exposure times: using chemiluminescence substrates for detection and signals are recorded on X-ray films after 5-15 min exposure time allowing multiple exposures from a single blot. There were no quantitative changes in the protocol of the Photogene System but the quality of the washing process was improved by the post hybridization, blocking and detection steps. There is an economic advantage when using the Photogene System if the cost for reaction is analyzed.

It is recommend the application of the chemiluminescent technique for genetic characterization of *S. tritici* isolates in labs. where radioactivity labeling is not allowed because the images obtained with this technique have the same quality to that obtained with radioactive techniques.

ACKNOWLEDGMENTS

We wish to thank the authorities of CIC and CONICET: PICT 97, PICT 99 (08-06356[a]) for the financial support, Natalia Kripelz and Silvina Larrán for their technical assistance and all the people who assisted us to collect materials and work at the lab.

REFERENCES

1. Brokenshire, T., 1975. Wheat seed infection by *Septoria tritici*. Trans. Br. Mycol. Soc., 64: 331-335.
2. Eyal, Z., A.L. Scharen, M.D. Huffman and J.M. Prescott, 1985. Global insights into virulence frequencies of *Mycosphaerella graminicola*. Phytopathol., 75: 1456-1462.
3. McDonald, B.A. and J.P. Martínez, 1990. DNA restriction fragment length polymorphism among *Mycosphaerella graminicola* (anamorph *Septoria tritici*) isolates collected from a single wheat field. Phytopathol., 80: 1368-1373.
4. McDonald, B.A. and J.P. Martínez, 1990. Restriction fragment length polymorphisms in *Septoria tritici* occur at a high frequency. Curr. Gen., 17: 133-138.
5. Boerger, J.M, R.S. Chen and B.A. McDonald, 1993. Gene flow between geographic populations of *Mycosphaerella graminicola* (Anamorph *Septoria tritici*) detected with restriction fragment length polymorphism markers. Phytopatol., 83: 1147-1154.
6. McDonald, B.A. and J.P. Martínez, 1991. Chromosome length polymorphisms in *Septoria tritici* population. Curr. Gen., 19: 265-271.
7. McDonald, B.A., R.E. Pettway, R.S. Chen, J.M. Boerger and J.P. Martínez, 1995. The population genetics of *Septoria tritici* (teleomorph *Mycosphaerella graminicola*). Can. J. Bot., 73: 292-301.
8. McDonald, B.A., J. Zhan, K. Yarden, J. Hogan, J. Gardon and R.E. Pettway, 1998. The population genetics of *Mycosphaerella graminicola* and *Stagonospora nodorum.* In: Understanding pathosystem: A focus of Septoria. 15th Long Ashton International Symposium IACR-Long Ashton research Station, Bristol, England Chapter 3, pp: 44-69.
9. Shaw, M.W. and D.J. Royle, 1989. Airborne inoculum as a major source of *Septoria tritici* (*Mycosphaerella graminicola)* infections in winter wheat crop in the UK. Plant Pathol., 38: 35-43.

10. Cordo, C.A. and H.O. Arriaga, 1990. Variación en patogenicidad entre cepas argentinas de *Mycosphaerella graminicola* (anamorfo Septoria *tritici*). In: Conferencia Regional sobre septoriosis del trigo. México, Cimmyt, D.F., M.M. Kohli, Y. Van and L.T. Beuningen (Eds.), pp: 88-100.
11. Cordo, C.A., A.E. Perelló, H.E. Alippi and H.O. Arriaga, 1990/91. Presencia de *Mycosphaerella graminicola* (Fuckel) Schroeter telemorfo de *Septoria tritici* Rob. Apud Desm. en trigos maduros de la Argentina. Rev. Fac. Agron. Tomo, 66/67: 49-55.
12. Cordo, C.A., A.E. Perelló, H.O. Arriaga and A. Pascar, 1989. Study of the stability of *Mycosphaerella graminicola* (*Septoria tritici*) isolates. In: Septoria of Cereals. Proc. 3rd Intl. Workshop Zurich, Switzerland. Fired, P.M. (Eds.), pp: 55-59.
13. Cordo, C.A., A.E. Perelló, H.O. Arriaga, G. Benedicto, V. Avila and I.R. Ziglino, 1994. Resistencia a la mancha foliar causada por *Septoria tritici* en trigo pan (*Triticum aestivum*). Rev. Fac. Agron., 70: 23-36.
14. Perelló, A.E., F.J. Babinec and C.A. Cordo, 1990. Especialización fisiológica en cepas argentinas de *Mycosphaerella graminicola* (Fuckel) Schroeter (Anamorfo). *Septoria tritici* Rob. ex Desm. In: Conferencia Regional sobre Septoriosis del trigo. Cimmyt, F., M.M. KohLI, Y. Van and L.T. Beuningem (Eds.), México, D.F., pp: 101-107.
15. Perelló, A., C.A. Cordo, H.O. Arriaga and H.E. Alippi, 1991. Variation in virulence in isolates of *Septoria tritici* Rob. ex Desm. on wheat. Agron., Omie, 11: 571-579.
16. Large, E.C., 1954. Growth stages in cereals, illustration of the Feekes scale. Plant Pathol., 3: 128-129.
17. Anonymous, 1968. Plant Pathologist´s Pocketbook. The Commonwealth Mycological Institute, C.A.B. Kew Surrey England, pp: 267.
18. Biochemica, 1992. Application Manual DIG DNA Labeling and Detection Nonradioactive. Boehringer Mannheim, Germany, pp: 62.
19. Hegnaver, R., 1971. Digitalis purpurea and *Digitalis lanata*: Good source of digoxigenina. Naturwiss, 58: 585-598.
20. Reed, C.K. and D.A. Mann, 1985. Rapid transfer of DNA from agarose gel to nylon membranes. Nuclei. Acids. Res., 13: 7207-7221.
21. Bioprime DNA Labelling System., 1998. Life Technologies Inc. Cat.N°18094-011. Photogene Nucleic Acid Detection System Version 2.0 Instruction Manual. Life Technologies, Cat.N°18092-057 Inc., USA, pp: 26.
22. Mc Dermott, J.M. and B.A. McDonald, 1993. Gene flow in plant pathosystems. Annual Rev. Phytopathol., 31: 353-373.
23. Cordo, C.A., M.R. Simón, A.E. Perelló and H.E. Alippi, 1999. Spore dispersal of leaf blotch pathogens of wheat (*Mycosphaerella graminicola* and *Septoria tritici*). In: Septoria and Stagonospora Diseases of Cereals: a compilation of global research. México: Cimmyt, D.F., M.A. Van Ginkel, A. McNab and J. Krupinsky (Eds.), pp: 98-101.