DEVELOPMENT OF SSR & STS MOLECULAR MARKERS ASSOCIATED WITH STEM RUST RESISTANCE IN BREAD WHEAT (*Triticum aestivum* L.)

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Wheat (*Triticum aestivum* L.) is the most important strategic cereal crop for the majority of the world populations. It is the one of most important staple food for about two billion people (36% of the world population). It exceeds in acreage and production other grain crops (including rice, maize, etc.).

Wheat is an edible grain, one of the oldest and most important cereal crops in Egypt. The annual consumption of wheat grains in Egypt is about 12.4 million tons, while the annual local production is about 9.38 million tons/ 3.4 million faddan in 2014/2015 (Agric. Economics and Statistics Department, Ministry of Agriculture, Egypt, 2015). The required yield increase may be achieved by developing high-yielding cultivars a long with implementing improved cultural practices. The new improved cultivars must be resistant to serious diseases such as wheat rusts, tolerant to abiotic stresses namely; drought, salinity and heat, and should be genetically stable in a broad spectrum of environments (having wide adaptability). Therefore, the efforts of wheat breeders and geneticists must continue to increase the productivity per unit area to narrow the gap between supply and consumption in Egypt.

Stripe, leaf and stem rusts caused by *Puccinia striiformis, Puccinia triticinea* and *Puccinia graminis,* respectively, are globally important wheat fungal diseases that cause significant grain yield losses. Use of resistant wheat cultivars is the most economic and environmentally safe approach to reduce crop losses from rust diseases. However, understanding the genetic behavior of wheat resistance to these diseases is essential for deciding the breeding strategies that maximize the genetic improvement of these traits (Shehab El-Din *et al.*, 1991).

Wheat resistance to rusts has been assumed to be a relatively simple inherited trait (Biffen, 1905) governed by one, two or few number of major genes (Dyck, 1991; Bai *et al.*, 1997). Meanwhile, several investigators indicated that resistance is a quantitative character controlled by many genes as well as the prevailing environmental conditions (Shehab El-Din *et al.*, 1991; Yadav *et al.*, 1998; Nawar *et al.*, 2010). Furthermore, resistance was dominant over susceptibility in most cases

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(Shehab El-Din and Abd El-Latif, 1996; Bai *et al.*, 1997; Patil *et al.*, 2000) while others claimed an opposite concept (Singh *et al.*, 1998; Ganeva *et al.*, 2001). On the other hand, some reports fit a simple additive genetic model with no dominance or epistatic interactions, while dominance and/or epistasis were more pronounced and had important roles (Shehab El-Din and Abd El-Latif, 1996; Singh *et al.*, 1998; Nawar *et al.*, 2010).

Molecular markers are useful tools to study genetic variations, since the genetic variability among wheat varieties is narrow as in all self-pollinated crops (Röder *et al.*, 2002). The applications of molecular markers in plant breeding programs facilitate the improvement of many crop species (Williams *et al.*, 1990). It offers the simplest and fastest method for detecting a great number of genomic markers in a short period of time (Edwards *et al.*, 1992). Michelmore *et al.* (1991) developed the F_2 plants population to the highest and the lowest extremes for the development of markers needed for marker-assisted selection. Marker-assisted selection was successfully practiced in several crop plants such as rice (Naqvi *et al.*, 1995), wheat (Penner *et al.*, 1996), durum wheat (Wang *et al.*, 1995), rapeseed (Jourdren *et al.*, 1996) and maize (Abdel-Tawab *et al.*, 1998).

The objectives of this study were to screen the response of twelve bread wheat genotypes under infection condition with respect to their performances to select the most resistant and the most susceptible varieties or lines, test stem rust on the contrasting parents and their F_1 and F_2 plants by recording the rust reaction and some related traits to stem rust and detect some molecular genetic markers associated with stem rust using SSR & STS markers.

MATERIALS AND METHODS

1. Materials

This study was carried out in the research farm and the laboratory of the Wheat Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Department of Genetics, Faculty of Agriculture, Ain Shams university and laboratories of INRA, Rabat, Morocco, during the period from 2010 to 2015.

Three bread wheat genotypes (*Triticum aestivum* L.) namely; Misr1 (resistant to stem rust), Line 37 (susceptible to stem rust) and Line 92 (susceptible to stem rust) were chosen from a preliminary screening trial for stem rust resistance which comprised twelve bread wheat genotypes according to their resistance to stem rust. The grains of 12 wheat genotypes were kindly obtained from the Wheat Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt as listed in Table (1).

2. Methods

The pedigrees and origins of the three selected genotypes (Misr1, Line 37

and Line 92) are shown in Table (2), they were grown in the field and crossed (Misr1 x line 37, Misr1 x Line 92 and line 37 x line 92) to obtain the F_1 grains for these three crosses. Some of the F_1 grains for each cross were sown in the field and selfed to obtain the F_2 grains.

2.1. Evaluation of the parent, (F_1) plants and (F_2) individual plants for each hybrid under normal and infected conditions in the field

Parents, F_1 plants for the three crosses were grown at Gemmeiza Research Station in three replications in a randomized complete block design experiment and 200 F_2 individual plants for each cross were cultivated in the optimum planting date (normal conditions) and in the late planting date (infection conditions).

Data were recorded for all plants at the end of the experiment for the following yield-related traits related to stem rust; days to heading, days to maturity, plant height (cm), number of spikes/plant, spike length (cm), number of spikelets/spike, grain yield/plant (g) and rust reaction.

2.2. Statistical analysis

The collected data from the three crosses (parents and F_1 plants were statistically analyzed using analysis of variance (ANOVA) procedure according to Snedecor and Cochran (1969).

The F_2 plants, represented by 200 plants for each cross were classified according to their behavior under infection conditions. According to their performances (rust reaction), eight resistant F_2 individual plants and eight susceptible F_2 individual plants for Misr1 X Line 37 hybrid, seven resistant F_2 individual plants and five susceptible F_2 individual Plants for Misr1 X Line 92 hybrid and seven resistant F_2 individual plants and eight susceptible F_2 individual plants for Line 37 X Line 92 hybrid were chosen for further molecular analysis with their parents and F_1 plants.

2.3. Molecular genetic analysis

2.3.1. Genomic DNA extraction

DNeasy™ Plant Mini Kit (Qiagen Inc., cat. No. 69104) was used for DNA isolation as described by the manufacturer manual from plant samples, i.e., the three parents, their F_1 plants and the most resistant F_2 individual plants as well as the most susceptible F_2 individual plants for each cross (Dellaporta *et al*., 1983).

2.3.2. SSR & STS markers by PCR-based analysis

PCR reactions were performed according to Williams *et al.* (1990) using six SSR & STS specific primers (Operon Technology, USA) as shown in Table (3). The reaction conditions were optimized and mixtures (10 µl total volume) were composed of dNTPs (1 µl), 5X green buffer (2 µl), $MgCl_2$ (0.6 µl), primer (1 µl), DNA (1 µl), Taq DNA polymerase (0.06) and H_2O up to 10 µl.

Amplification was carried out in a Primus Thermocycler, programmed for 37 cycles as follows: denaturation, 94°C/5 min (one cycle); annealing, 94°C/30 sec; 59°C/30 sec; 72°C/45 sec (35 cycles); extension, 72°C/5 min (one cycle); then 4°C until use. Agarose gel (1.2%) and acrylamide gel (8%) electrophoresis were used for separating the PCR products. The run was performed at 100 volts for about one hour. DNA Marker used in this study was 1 and 1.5 kb DNA ladder.

2.3.3. Analysis of gel images

All fragments resulting from polyacrylamide and agarose gels were detected on an UV-transilluminator filter. All gels were photographed under UV light with Polaroid film 667 and scanned with Bio-Rad video densitometer Model 620 at a wavelength of 577. Appropriate software was used for data analysis.

RESULTS AND DISCUSSION

1. Stem rust-related traits

1.1. Response of the parents and F_1 plants

The means of stem rust-related traits of the three parents and F_1 plants for each one under normal and infected conditions are shown in Table (4). Infection condition (late planting date) caused reductions in the estimates of all traits except spike length and number of spikelets per spike traits.

Plant height trait values showed reductions in all genotypes under infection condition which were lower than all genotypes under normal condition (optimum planting date), except the F_1 plants for cross 3 (line 37 x line 92) which showed the same value under both conditions. Moreover, line 37 displayed the highest reduction value, while line 92 showed the lower reduction value. These results agreed with those reported by Khattab (2009) and Darwesh (2011).

With respect to number of spikes per plant trait, the resistant parent (Misr1) and the F_1 plants for cross 2 (Misr1 x Line 92) exhibited a higher number (elven spikes per plant) than the two susceptible parents (Line 37 and Line 92) under infection condition, the F_1 plants for cross 1 (Misr1 x Line 37) and the F_1 plants for cross 3 (Line37 x Line 92) which were 9, 10, 8 and 9, respectively, under infection condition. The F_1 plants for cross 2 (Misr1 x Line 92) showed the same value under normal and infection condition which was 11 spikes/ plant. Comparable results were reported by Talbert *et al.* (2001) and Hendawy *et al.* (2009).

For grain yield per plant trait, there were sharp decreases in the values of the F_1 plants for cross 3 (Line 37x Line 92), the susceptible parent Line 37, the susceptible parent Line 92, the F_1 plants for cross 2 (Misr1 x Line 92) and the F_1 plants for cross 1 (Misr1 x Line 37) under infected condition (23.32, 23.85, 25.78, 32.99 and 34.72, respectively) compared with the normal condition (47.54, 44.69, 49.5, 47.41 and 45.52, respectively). While the resistant parent (Misr1) recorded the high-

est value in grain yield under infection condition (46.91) compared with normal condition (49.39), which indicated that this resistant parent could relatively resist to stem rust disease. These results are in agreements with those of Tammam (2005) and El-Hawary (2010).

Days to heading and days to maturity traits showed lower values in all genotypes under infection condition compared with all genotypes under normal condition, the susceptible parent (Line 37) showed the sharpest reduction between days to heading under normal and infection conditions (107 and 97, respectively), on the other hand the susceptible parent (Line 92) showed the highest reduction between days to maturity under normal and infection conditions (154 and 144, respectively). Similar results were obtained by Talbert *et al.* (2001), Akhter *et al*. (2003) and Hendawy *et al.* (2009).

Spike length trait (Table 4) showed a slight difference between the normal and infection conditions for all genotypes. The resistant parent (Misr1) under normal condition and the F_1 plants for cross 2 (Misr1 x Line 92) under normal and infection condition, value (12 cm) was longer than all other genotypes. The susceptible parent (line 92) showed the lowest value (10 cm) under normal and infection conditions compared with other parents and their F_1 plants. These results agreed with those reported by Khattab (2009) and El-Hawary (2010).

For number of spikelets per spike trait, there was a slight difference between the normal and infection conditions for all genotypes. These results are in agreement with Tammam (2005)

1.2. Performance of F_2 plants

F_2 plants, represented by 200 individuals for each cross, were classified into groups according to their performances under infection condition for each trait. Then, rust reaction, plant vigor and grain yield traits classified the F_2 plants into groups for the three crosses. The first group refers to the best growing F_2 plants that were resistant to stem rust and high yielding under infection condition and the last group refers to the worst ones that were susceptible to stem rust and low yielding under infection condition. The F_2 plants were arranged in descending order according to their frequency. Plants with high frequency in the first group were chosen as the most resistant F_2 plants. While, plants in the last group were taken to represent the most susceptible F_2 plants.

According to these classifications, eight F_2 individual plants were selected to represent the most resistant F_2 plants and eight plants were chosen as the most susceptible ones to stem rust in cross 1 as shown in Table (5). Seven F_2 individual plants were selected to represent the most resistant F_2 plants and five plants were chosen as the most susceptible ones to stem rust in cross 2 as shown in Table (6). Seven F_2 individual plants were selected to represent the most resistant F_2 plants (these plants were escaped from stem rust infection, because the two parents for this hybrid are susceptible to stem rust) and

eight plants were chosen as the most susceptible ones to stem rust in cross 3 as shown in Table (7).

These F_2 resistant plants and F_2 susceptible plants were used as individual plants to obtain SSR and STS markers associated with stem rust.

Many authors evaluated contrasting parents and their segregated F_2 population plants to detect some molecular markers associated with abiotic and biotic stresses as well as yield component and quality traits in plants. However, their results reflected significant differences between parental genotypes for the studied trait(s) which indicated the presence of variability between these parents. Moreover, the segregated F_2 population plants were classified to the highest and the lowest groups based on the studied trait(s) to develop molecular markers. Thus the resulting resistance genes in the F_2 populations must be inherited from the resistant parent (Haley *et al.*, 2008; Onweller, 2011). In this respect, Rashed *et al.* (2006) evaluated some salt tolerance-related traits in sorghum, Atta *et al.* (2006) recorded some iron deficiency-related traits in maize, McIntosh *et al.* (1995) found that many of the introgressed genes are also associated with undesirable effects on agronomic traits. Michelmore *et al.* (1991) identificatied some molecular markers linked to disease-resistance genes by bulked segregant analysis. Although, several markers were reported as tightly linked to target resistance genes in a specific population in previous studies, they were not diagnostic when in different backgrounds.

2. SSR and STS markers for stem rust resistance

DNA isolated from Misr1 as a stem rust resistant parent, Line 37 and Line 92 as susceptible parents, their subsequent F_1 plants, and the F_2 segregated population (the most resistant and the most susceptible individual plants) for the three crosses were tested against six preselected SSR & STS specific primers as shown in Figs. (1, 2 and 3) and summarized in Tables (8, 9 and 10).

Only two primers (*Sr2* and *Sr25*) detected positive molecular markers for stem rust resistance with the studied genotypes in crosses 1 and 2, while the other four primers failed to develop molecular markers for stem rust resistance as shown in Tables (8 and 9). *Sr2* primer exhibited a positive molecular marker with molecular size of 120 bp which was found only in the resistant parent Misr1, the F_1 plants and the most resistant F_2 individual plants, while they were absent in the susceptible parents (Line 37 for cross 1 and Line 92 for cross 2) and the most susceptible F_2 individual plants (five plants for cross1 and three plants for cross 2).

Sr25 primer detected a positive molecular marker with molecular size of 130 bp which was found only in the resistant parent Misr1, the F_1 plants and the most resistant F_2 individual plants, while they were absent in the susceptible parents

(Line 37 for cross 1 and Line 92 for cross 2) and the most susceptible F_2 individual plants (six plants for cross 1 and three plants for cross 2).

Consequently, *Sr2* and *Sr25* loci at fragment sizes of 120 and 130 bp, respectively, were apparently associated with stem rust resistance according to the presence of them in cross 1 (RP x SP1) and cross 2 (RP x SP2) as Misr1 (the resistant parent) was included in each one of them. Moreover, these two loci were present in the resistant F_2 groups, while only one locus of these two loci were observed in some individuals of the susceptible F_2 groups of these two crosses due to the contribution of the resistant parent (Misr1). On the other hand, these two loci were actually absent in cross 3 (SP1 x SP2) which included the two susceptible parents. In addition, *Sr38* locus at fragment size of 262 bp was present in cross 1 (RP x SP1) due to the contribution of the susceptible parent Line 37 (SP1) as well as in cross 3 (SP1 x SP2), while it was absent in cross 2 (RP x SP2). Finally, *Sr24* locus at fragment size of 200 bp was a common fragment in all crosses, while *Sr36* and *Sr39* loci at fragment sizes of 155 and 900 bp, respectively, were absent in all crosses but they appeared in monogenic lines *Sr36* and *Sr39*, which means that these three loci were not associated with stem rust resistance. Therefore the resistant parent, their F_1 plants and most resistant F_2 individual plants which were resistant to stem rust exhibited both *Sr2* and *Sr25*.

These two positive markers could be considered as reliable markers for stem rust resistance in bread wheat. These results agreed with many reports which detected molecular markers for biotic stresses resistance. Molecular markers are available for only few resistance genes such as *Sr2* (Hayden *et al*., 2004), *Sr24* (Mago *et al*., 2005), *Sr36* (Bariana *et al*., 2001; Tsilo *et al.*, 2008) and *Sr39* (Gold *et al.*, 1999). Abdel-Tawab *et al.* (2003) detected five positive and negative RAPD markers for drought tolerance in Egyptian bread wheat. Some of these markers have been used in MAS (Marker assisted selection). At the present time, the research of stem rust in wheat is focusing on identifying more resistance genes to stem rust.

Moreover, our results were in agreement with those of Nachit *et al.* (2000) who associated yield-related traits as grain yield, yield components and stress physiological traits with some molecular markers in durum wheat. Several markers showed strong relationships with grain yield, yield components and stress physiological traits, indicating that there are potential markers for use in marker-assisted selection to improve biotic stresses resistance known as molecular breeding.

SUMMARY

Screening experiment was performed on twelve genotypes of bread wheat *(Ttriticum aestivum* L.*)* to select the most stem rust resistant genotype (Misr1) and the most stem rust susceptible genotypes (Line 37 and Line 92) according to stem rust reaction. Crosses were carried

out between the resistant parent (Misr1) with each of the susceptible parents as well as between the two susceptible parents (Line 37 and Line 92) to obtain the F_1 kernels. Some of the F_1 kernels were sown in the field and selfed to obtain the F_2 kernels for each cross. These three selected parents, their F_1 and the most resistant and susceptible F_2 plant groups for the three crosses were evaluated for their response to stem rust resistance by recording some stem rust–related traits. However, infected condition caused a reduction in the values of all traits except spike length and number of spikelets per spike traits. The three parents, their F_1 plants and some individual plants of the two contrasting F_2 plant groups (the most resistant and the most susceptible F_2 groups) for the three crosses were used to develop some molecular genetic markers associated with stem rust resistance using SSR and STS markers. The results indicated the presence of two positive markers out of the three SSR and three STS primers which used in this study. *Sr2* (SSR) and *Sr25* (STS) primers gave positive markers at fragment sizes of 120 and 130 bp, respectively, for stem rust resistance that could be considered as reliable markers for stem rust resistance in bread wheat (*Ttriticum aestivum* L.).

REFERANCES

Abdel-Tawab, F. M., C. W. Stuber, M. A. Rashed, A. Bahieldin and A. H. Atta (1998). Genome mapping and detection of QTLs linked with salt tolerance in maize. Proceed. Int. Conf. Mol. Genet., 230-248.

Abdel-Tawab, F. M., Eman M. Fahmy, A. Bahieldin, Asmahan A. Mahmoud, H. T. Mahfouz, Hala F. Eissa and O. Moseilhy (2003). Marker assisted selection for drought tolerance in Egyptian bread wheat (*Triticum aestivum* L.). Egypt. J. Genet. Cytol., 32: 34-65.

Agric. Economics and Statistics Department, Ministry of Agriculture, Egypt (2015).

Akhter, Z., A. K. M. Shamsuddin, M. M. Rohman, M. S. Uddin, M. Mohi-Ud-din and A. K. M. M. Alam (2003). Studies on heterosis for yield and yield components in wheat. J. Bio. Sci., 10, 3: 892-897.

Atta, A. H., M. A. Rashed, M. S. Abdel-Hady and Hoda M. H. Elnaggar (2006). Marker-assisted selection for iron deficiency tolerance in maize. J. Biol. Chem. Environ. Sci., 1: 205-224.

Bai, D., D. R. Knott and J. M. Zale (1997). The inheritance of leaf and stem rust resistance in *Triticum monococcum* L. Can. J. Plant Sci., 78: 223-226.

Bariana, H. S., M. J. Hayden, N. U. Ahmed, J. A. Bell, P. J. Sharp and R. A. McIntosh (2001). Mapping of durable adult plant and seedling resistances to stripe rust and stem rust diseases in wheat. Aust. J. Agric. Res., 52: 1247-1255.

Biffen, R. H. (1905). Mendel's laws of inheritance and wheat breeding. J. Agric. Sci., 1: 4-48.

Darwish, M. A. H. A. (2011). Genetical studies on some rusts in bread wheat Ph.D. Thesis, Fac. of Agric, Tanta, Univ., Egypt.

Dellaporta, S. L., J. Wood and J. B. Hicks (1983). A plant DNA mini preparation. Version III. Plant Mol. Biol., 1: 19-21.

Dyck, P. L. (1991). Genetic of adult plant leaf rust resistance in Chinese spring and Sturdy wheat. Crop Sci., 31: 309-311.

Edwards, M. D, T. T. Helntijaris, S. Wright and C. W. Stuber (1992). Molecular marker facilitated investigation of quantitative trait loci in maize. 4. Analysis based on genome saturation with isozyme and restriction fragment length polymorphism. Theor. Appl. Genet., 83: 765-74.

El-Hawary, M. N. A. (2010). Breeding for stress tolerance in some bread wheat. Ph. D. Thesis, Fac. of Agric, Mansoura Univ., Egypt.

Ganeva, G., M. Todorova and H. Kurzhin (2001). Inheritance of the resistance to the causative agent of the brown rust in wheat varieties and lines. Rasteniev" dni Nauki 38: 181-185. (C.F. Review of Plant Patho. 81: 9352).

Gold, J., D. Harder, F. Townley-Smith, T. Aung and J. Procunier (1999). Development of a molecular marker for rust resistance gene *Sr39* and *Lr35* in wheat breeding lines. EJB Elect. J. Biotech., 2.

Haley, S. D., J. J. Johnson, F. B. Peairs, J. S. Quick, J. A. Stromberger, and J. D. Butler (2008). Registration of 'Bill Brown' wheat. Journal of Plant Registrations, 2: 218-223.

Hayden, M. J., H. Kuchel, and K. J. Chalmers (2004). Sequence tagged microsatellites for the Xgwm533 locus provides new diagnostic markers to select for the presence of stem rust resistance gene *Sr2* in bread wheat *(Triticum aestivum* L.). Theor., Appl. Genet., 109: 1641-1647.

Hendawy, F. A., H. A. Dawwam, M. A. Abo Shereif and E. L. El-Massry (2009). Detection of epistasis in the inheritance of grain yield and its components in bread wheat. Minufiya J. Agric. Res., 34: 625-640.

Jourdren, C., P. Barret, R. Horvais, N. Foisset, R. Delourme and M. Renard (1996). Identification of RAPD markers linked to the loci controlling erucic acid level in rapeseed. Mol. Breeding, 2: 61-71.

Khattab, A. B. (2009). Genetic behavior of some traits of bread wheat (*Triticum aestivum* L.). J. Agric.

Res. Kafr El-Sheikh Univ., 35: 446-479.

Mago, R., H. S. Bariana, I. S. Dundas, W. Spielmeyer, G. J. Lawrence, A. J. Pryor and J. G. Ellis (2005). Development of PCR markers for the selection of wheat stem rust resistance genes *Sr24* and *Sr26* in diverse wheat germplasm. Theor., Appl. Genet., 111: 496-504

McIntosh, R. A., C. R. Wellings and R. F. Park (1995). Wheat Rusts: An Atlas of Resistance Genes.

Michelmore, R. W., I. Paran and R. V. Kessel (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA, 88: 9829-9832

Naqvi, N. I., J. M. Bonman, D. J. Mackill, R. J. Nelson and B. B Chattoo (1995). Identification of RAPD markers linked to a major blast resistance gene in rice. Mol. Breeding, 1: 341-348.

Nachit, M. M, P. Monneveux, M. E. Sorrells, C. Royo, N. Fonzo and J. L. Araus (2000). Relationship of dry-land productivity and drought tolerance with some molecular markers for possible MAS in durum *(Triticum turgidum* L. *var. durum*). Durum wheat improvement in the Mediterranean region: new challenges. Proceedings of a Seminar, Zaragoza, Spain, 40: 203-206.

Nawar, A. A., T. M. Shehab El-Din, A. N. Khalil, H. H. Nagaty and K. E. Ragab (2010). Inheritance nature of leaf rust resistance and some agronomic characters in bread wheat. J. of Plant Production, 1: 417-429.

Onweller, K. M. (2011). Disease and insect resistance and quality characterization of six CIMMYT synthetic hexaploid wheats. Unpublished M.Sc., University of Nebraska-Lincoln. Retreived May 20, 2012, from http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1031&context=agronhort diss

Patil, J. V., A. B. Deokar and R. B. Deshmukh (2000). Genetic analysis of three wheat cultivars for reaction to stem rust of wheat. Indian J. Agric. Res. 34: 275-277. (C.F. Review of Plant Pathology 81(9): 8192).

Penner, G. A., S. J. Lee, L. J. Bezte and E. Ugali (1996). Rapid RAPD screening of plant DNA using dot blot hybridization. Molecular Breeding, 2: 7-10.

Rashed, M. A., A. Abo-Doma, H. El-Rashidy and K. Khaled (2006). Molecular genetic characterization for some loci controlling salt tolerance in *Sorghum bicolor* (L).

Egypt. J. Genet. Cytol., 35: 145-155.

Röder, M. S., K. Wendehake, V. Korzun, G. Bredemeijer, D. Laborie, L. Bertand, P. Isaac, S. Rendell, J. Jackson, R. J. Cooke, B. Vosman, and M. W. Gamal (2002). Construction and analysis of a microsatellite-based database of European wheat varieties. Theor. Appl. Genet., 95: 714-722.

Shehab El-Din, T. M., M. A. Gouda, S. Abouel-Naga and M. M. EL-Shami (1991). Quantitative study on wheat resistance to stem rust caused by *Puccinia graminis tritici*. J. Agric. Sci. Mansoura Univ., 16: 1298-1303

Shehab El-Din, T. M. and A. H. Abd El-latif (1996). Quantitative determination of the gene action of stripe rust resistance in a 6-parent diallel cross of wheat. J. Agric. Sci. Mansoura Univ., 21, 10: 3461-3467.

Singh, R. P., A. Mujeeb-Kazi and J. Huerta-Espino (1998). *Lr46:* a gene conferring slow-rusting resistance to leaf rust in wheat. Phytopathol., 88: 891- 894.

Snedecor, C. W. and W. G. Cochran (1969). Statistical methods 6th Ed. Lowa State Univ. Press, Ames, lowa, USA.

Talbert, L. E., S. P. Lanning, R. L. Murphy and J. M. Martin (2001). Grain filling duration in twelve hard red spring wheat crosses: genetical variation and association with other agronomic traits. Crop Sci., 41: 1390-1395.

Tammam A. M. (2005). Generation mean analysis in bread wheat under different environmental conditions. Minufiya J. Agric. Res., 30: 937-956.

Tsilo, T. J., Y. Jin and J. A. Anderson (2008). Diagnostic microsatellite markers for the detection of stem rust resistance gene *Sr36* in diverse genetic backgrounds of wheat. Crop Sci., 48: 253-261.

Wang, R. R. C., J. Chen and L. R. Joppa (1995). Production and identification of chromosome specific RAPD markers for Langdon durum wheat disomic substitution lines. Crop Sci., 35: 886-888.

Williams, J. G. K, A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey (1990). DNA polymorphisms amplified by arabitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.

Yadav, B., C. S. Tyagi and D. Singh (1998). Genetical studies and transgressive segregation for field resistance to leaf rust of wheat. Wheat Info. Serv., 87:15-21.

Table (1): Screening the responses of the twelve studied bread wheat genotypes under infected condition at the season of 2010-2011.

Ser. No.	Genotype	Stem rust "Rust reaction"	
1	Giza 168	tr Ms	Tris Moderately susceptible
2	Sakha 93	O	Escaped = Not Infected
3	Sakha 94	O	Escaped = Not Infected
4	Misr 1	R	Resistant
5	Gemmeiza 11	tr R	Tris Resistant
6	Sids 12	5S	5% Susceptible
7	Sakha 69	O	Escaped = Not Infected
8	Sids 1	O	Escaped = Not Infected
9	Attila*2 Giza 168	10S	10% Susceptible
10	Line37	20S	20% Susceptible
11	Soroca	10S	10% Susceptible
12	Line92	20S	20% Susceptible

Table (2): Name, pedigree and origin of the three selected parental genotypes.

Parent	Pedigree	Origin
Misr1 (P_1) (Resistant parent)	OASIS\SKAUZ\\4*BCN\3\2*PASTOR	ARC
Line 37 (P_2) (Susceptible parent 1)	THB//MAYA/NAC/3/RABE/4/MILAN	CIMMYT/ICARDA
Line 92 (P_3) (Susceptible parent 2)	SHUHA-1\PRINIA-31	CIMMYT/ICARDA

Table (3): List of Microsatellites (SSR) and Sequence Tagged Sites (STS) markers, chromosomes localization.

Microsatellite (SSR) markers		
Genes/Primers	Expected allel size	Chromosome location
Xgwm 533 Linked to *Sr 2*	120 bp	3B
Xstm 773 – 2 Linked to *Sr 36*	155 bp	2Bs
Sr 39/ Lr 35	900 bp	2B
Sequence Tagged Sites (STS) Markers		
Genes/Primers	Expected allel size	Chromosome location
Sr 38/Lr37/Yr 17	262 bp	2As
Sr 25 /Lr 19	130 bp	7A
Sr 24 / Lr24	200 bp	3 Dl

Table (4): Means of the recorded stem rust yield-related traits of the three contrasting parents and F_1 plants for each one.

Genotype	Condition	Days to Heading	Days to Maturity	Plant Height	No.of Spikes/ Plant	Spike Length	No.of Spiklets/ Spike	Grain Yield/ Plant (g)	Rust Reaction
Line 37	Normal	107	138	109	12	11	21	44.69	0
	Infection	97	131	95	9	11	21	23.85	S
F_1 (Misr 1 x Line 37)	Normal	103	135	106	9	11	21	45.52	0
	Infection	97	131	100	8	11	21	34.72	0, trs, 5S
Misr 1	Normal	99	133	106	14	12	22	49.39	0
	Infection	90	127	98	11	11	18	46.91	0
F_1 (Misr 1 x Line 92)	Normal	100	133	102	11	12	20	47.41	0
	Infection	98	131	100	11	12	20	32.99	0, trs
line 92	Normal	109	154	101	11	10	19	49.50	0
	Infection	101	144	100	10	10	18	25.78	S
F_1 (Line 37 x Line 92)	Normal	107	138	101	11	11	21	47.54	0
	Infection	102	132	101	9	11	21	23.32	20S, 10S, 5S

R = Resistant S = Susceptible O = Escaped Tris S = Tris Susceptible

Table (5): The performances of the most resistant & most susceptible F_2 (individual plants) for cross 1 (Misr 1 x Line 37) under infection condition (late planting date).

	Ser. No.	Plant No.	Plant Height	No.of Spikes/ Plant	Spike Length	No.of Spiklets/ Spike	Grain Yield/ Plant	Rust Reaction
The most resistant F_2 individual plants	1	10	92	27	11	19	52.3	R
	2	38	85	28	11	19	51.3	O
	3	55	100	28	11	19	50.2	O
	4	92	85	25	15	23	49.5	R
	5	142	103	19	11	21	48.3	O
	6	154	89	15	10	17	47.8	R
	7	181	100	25	12	21	46.4	R
	8	197	98	19	10	19	46.5	O
The most susceptible F_2 individual plants	1	1	93	25	10	21	25.7	10 S
	2	53	90	6	13	21	12.5	10 S
	3	99	101	7	11	21	18.3	5 S
	4	132	92	25	14	23	34.4	10 S
	5	139	102	10	10	21	19.6	5S
	6	162	103	9	10	21	16.5	10 S
	7	193	92	14	10	19	21.5	10S
	8	200	101	18	12	21	24.8	5 S

R = Resistant S = Susceptible O = Escaped

Table (6): The performances of the most resistant & most susceptible F_2 (individual plants) for cross 2 (Misr 1 x Line 92) under infection condition (late planting date).

	Ser. No.	Plant No.	Plant Height	No.of Spikes/ Plant	Spike Length	No.of Spiklets/ Spike	Grain Yield/ Plant	Rust Reaction
The most resistant F_2 individual plants	1	23	97	23	13	23	55.4	O
	2	77	86	23	10	21	53.4	R
	3	81	90	26	13	23	58.4	R
	4	90	99	25	12	21	53.2	R
	5	123	98	23	13	23	51.5	O
	6	159	89	26	12	21	53.7	R
	7	186	95	25	12	19	51.5	O
The most susceptible F_2 individual plants	1	2	88	8	10	19	7.7	5 S
	2	56	85	23	10	17	26.3	5 S
	3	79	98	14	9	19	14.1	10 S
	4	84	85	12	11	25	18.3	10S
	5	173	101	12	10	21	13.5	5 S

R = Resistant S = Susceptible O = Escaped

Table (7): The performances of the most resistant & most susceptible F_2 (individual plants) for cross 3 (Line 37 x Line 92) under infection condition (late planting date).

	Ser. No.	Plant No.	Plant Height	No.of Spikes/ Plant	Spike Length	No.of Spiklets/ Spike	Grain Yield/ Plant	Rust Reaction
The most resistant F_2 individual plants	1	1	93	23	13	23	55.3	O
	2	34	87	13	11	21	42.3	O
	3	47	86	16	9	19	48.5	O
	4	69	85	7	10	19	28.5	O
	5	85	91	10	12	21	34.5	O
	6	177	89	21	10	17	48.5	O
	7	180	83	22	10	19	52.2	O
The most susceptible F_2 individual plants	1	4	83	21	8	17	28.8	20 S
	2	17	83	25	11	19	23.6	30 S
	3	29	86	10	11	19	12.5	20 S
	4	32	81	10	13	23	13.2	20S
	5	78	87	5	12	19	10.7	30 S
	6	100	95	16	9	21	22.4	30 S
	7	139	93	18	11	21	22.8	20 S
	8	200	97	23	11	21	28.2	30 S

S = Susceptible O = Escaped

Table (8): Survey of the tested SSR & STS primers fragments with Misr 1 and Line 37 parents, their F_1 plants, the most resistant and most susceptible F_2 plants for cross 1 (Misr 1 x Line 37).

Primer Name	MS (bp)	RP	F1	SP1	R1	R2	R3	R4	R5	R6	R7	R8	S1	S2	S3	S4	S5	S6	S7	S8
Sr2	120	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	-	+	-
Sr24	200	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sr25	130	+	+	-	+	+	+	+	+	+	+	+	-	-	-	+	-	+	-	-
Sr36	155	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sr38	262	-	+	+	-	+	+	+	-	+	+	-	-	+	-	+	+	-	-	+
Sr39	900	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

RP=Misr1 SP_1=Line37 R1-R8=Resistant F_2 plants S1-S8=Susceptible F_2 plants MS=Molecular size

Table (9): Survey of the tested SSR & STS primers fragments with the Misr 1 and Line 92 parents, their F_1 plants, the most resistant and susceptible F_2 plants for cross 2 (Misr 1 x Line 92).

Primer Name	MS (bp)	RP1	F1	SP2	R1	R2	R3	R4	R5	R6	R7	S1	S2	S3	S4	S5
Sr2	120	+	+	-	+	+	+	+	+	+	-	+	-	-	-	+
Sr24	200	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sr25	130	+	+	-	+	+	+	+	+	+	+	-	+	-	+	-
Sr36	155	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sr38	262	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sr39	900	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

RP=Misr1 SP_1=Line92 R1-R7=Resistant F_2 plants S1-S5=Susceptible F_2 plants MS=Molecular size

Table (10): Survey of the tested SSR & STS primers fragments with the Line 37 and Line 92 parents, their F_1 plants, the most resistant and susceptible F_2 plants for cross 3 (Line 37 x Line 92).

Primer Name	MS (bp)	SP1	F_1	SP2	R1	R2	R3	R4	R5	R6	R7	S1	S2	S3	S4	S5	S6	S7	S8
Sr2	120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sr24	200	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sr25	130	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sr36	155	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sr38	262	+	+	-	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+
Sr39	900	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

SP_1=Line 37 SP_2=Line92 R1-R7=Resistant F_2 plants S1-S8=Susceptible F_2 plants MS=Molecular size

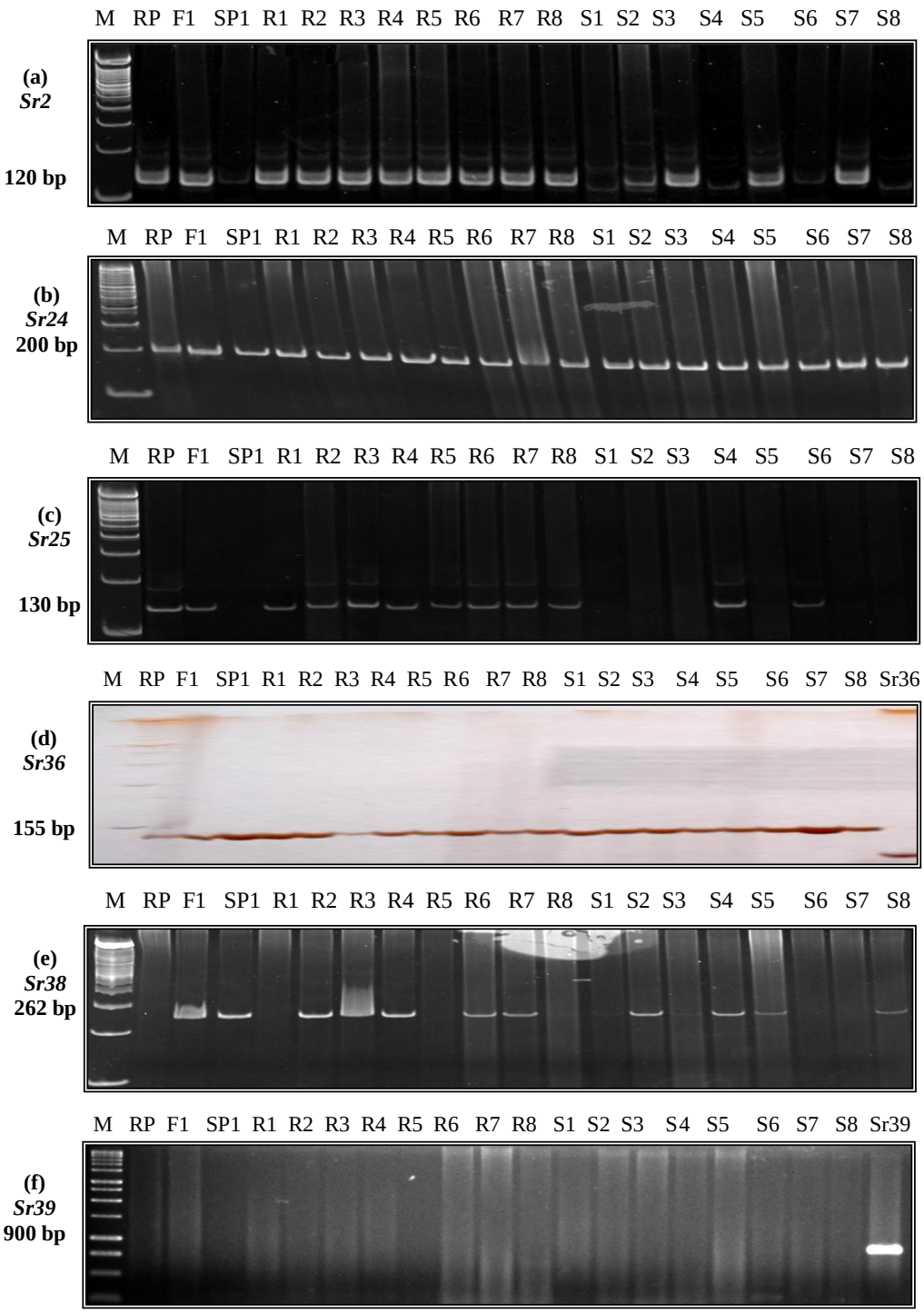

Fig. (1): SSR & STS fragments of six primers (*Sr2*, *Sr24*, *Sr25*, *Sr36*, *Sr38* and *Sr39*) for the resistant parent (RP_1), F_1 plants, the susceptible parent (SP_1), the most resistant F_2 plants (R_1….R_8), and the most susceptible F_2 plants (S_1….S_8) for cross 1 (Misr1 x Line37).

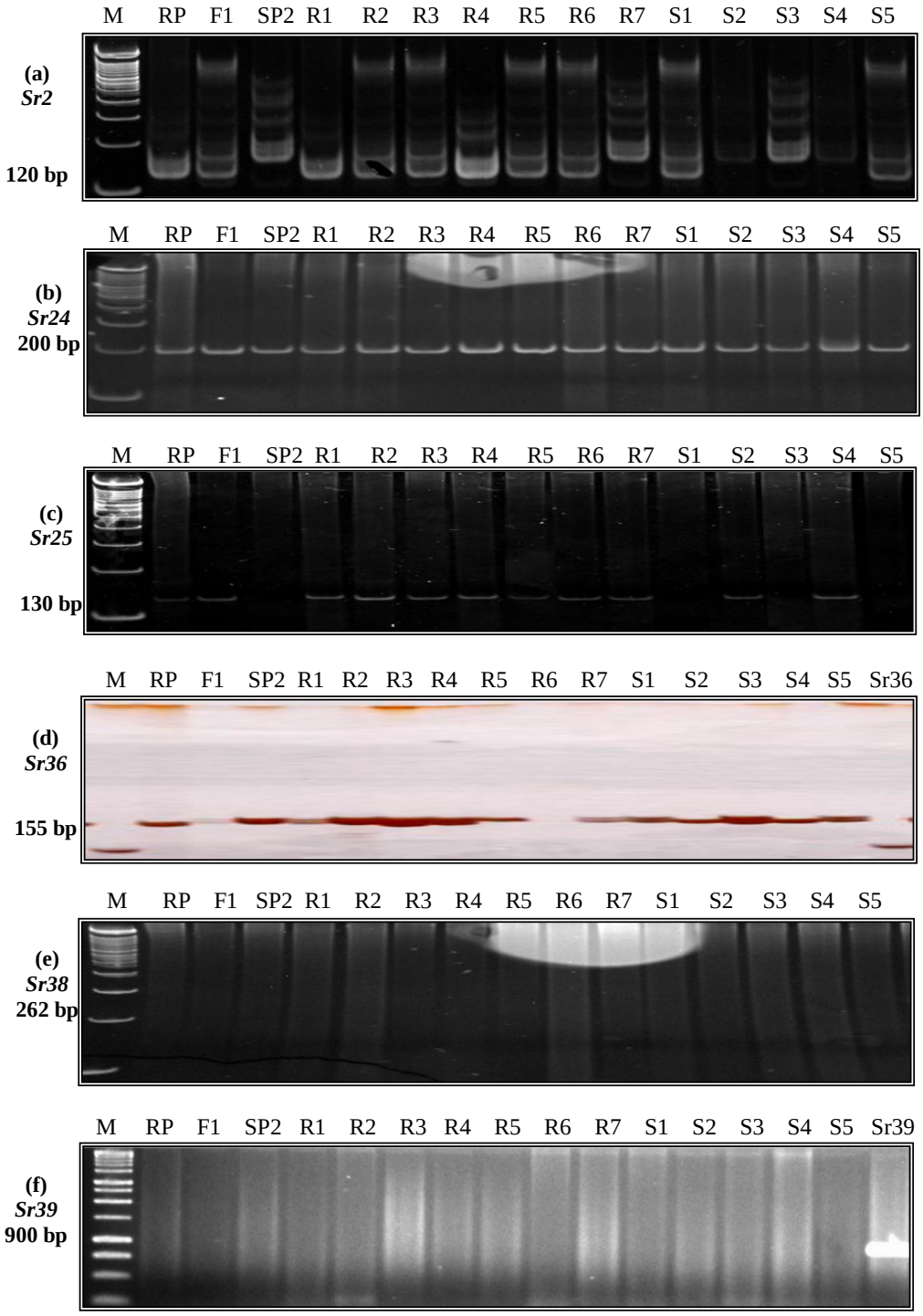

Fig. (2): SSR & STS fragments of six primers (*Sr2, Sr24, Sr25, Sr36, Sr38* and *Sr39*) for the resistant parent (RP_1), F_1 plants, the susceptible parent (SP_2), the most resistant F_2 plants (R_1….R_7), and the most susceptible F_2 plants (S_1….S_5) for cross 2 (Misr1 x Line92).

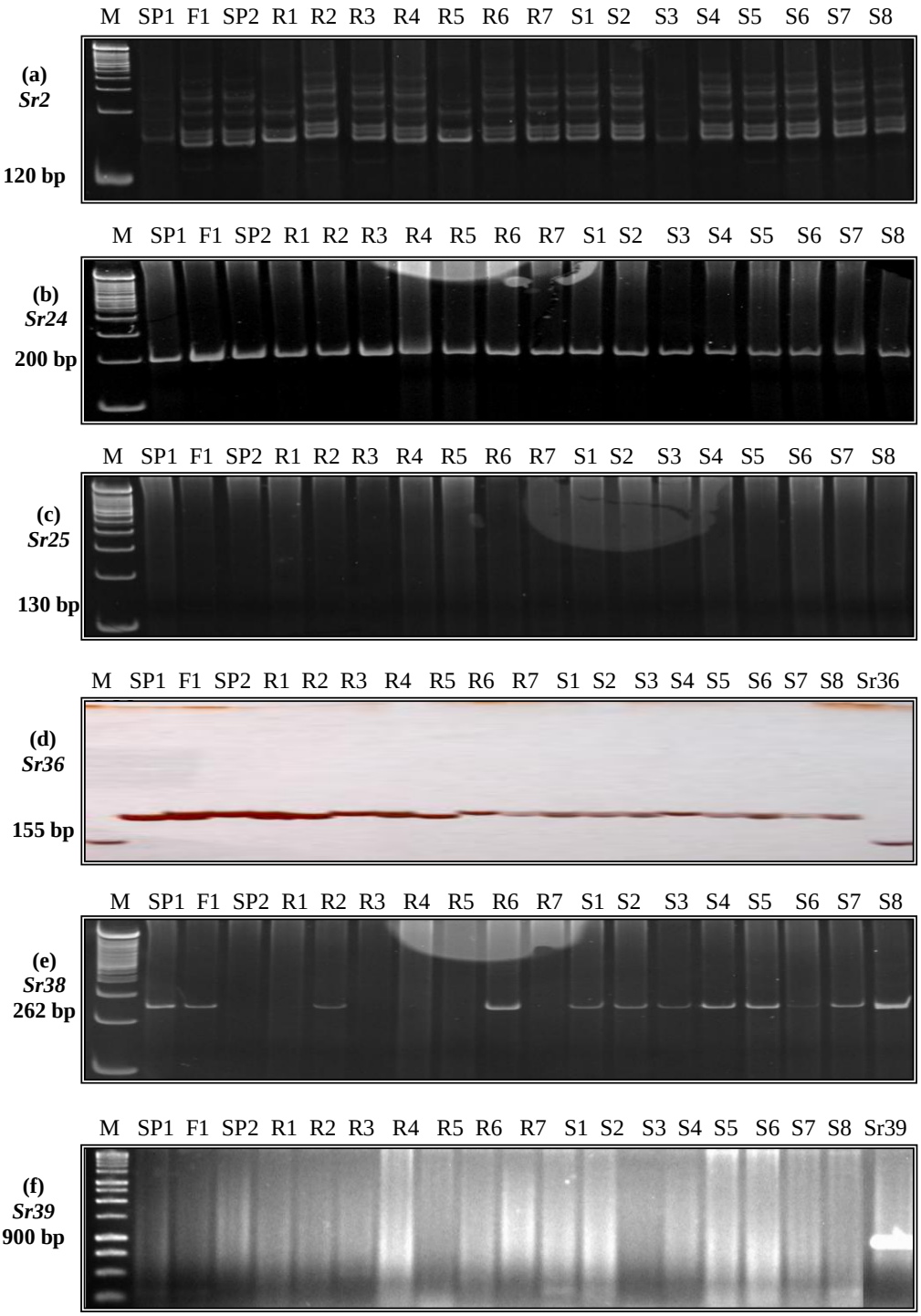

Fig. (3): SSR & STS fragments of six primers (*Sr2, Sr24, Sr25, Sr36, Sr38* and *Sr39*) for the resistant parent (SP_1), F_1 plants, the susceptible parent (SP_2), the most resistant F_2 plants (R_1….R_7), and the most susceptible F_2 plants (S_1….S_8) for cross 2 (Line 37 x Line92).