



Research Article

PCR-based characterization of *Puccinia graminis* f. sp. *tritici* race TTSSK from Iran

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Abstract: Stem rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) is one of the destructive diseases of wheat in the world. The fungal pathogen can infect 365 different grass and more than 70 *Berberis* species. DNA sequences for the ribosomal RNA internal transcribed spacer (ITS) have proven suitable to explore relationships at the species and subspecies levels. An isolate of *Pgt* which was collected from Iran and designated as TTSSK was used in this study. Three samples of the isolate were used. ITS region of the samples was amplified and sequenced. Consensus tree based on Maximum Parsimony clustering method was produced by Mega 6.0. Iranian isolate of TTSSK was placed in a clade with *P. graminis* which was isolated from *Berberis* sp. and *Pgt* isolate from Tajikistan. The results showed that more than one conserved genomic regions would be informative to identify phylogenetic relationship of Iranian *Pgt* isolates and samples from different parts of the world. Complementary studies with more sequence data from other genome loci are in progress.

Keywords: ITS, maximum parsimony, phylogenetic relation, stem rust, Ug99 race group

Introduction

Stem rust (black rust), caused by *Puccinia graminis* f.sp. *tritici* (*Pgt*) is one of the most important and destructive diseases of wheat in the world. The fungal pathogen is obligate parasite and can infect wheat plants during growing season (Singh *et al.*, 2006). The urediniospores can infect 365 different grass species including economically important crops like wheat, rye and oats (Leonard and Szabo, 2005). Also more than 70 *Berberis* species and some *Mahonia* species have been reported as alternate hosts of *Pgt* (Berlin *et al.*, 2012).

Emergence of Ug99 (TTKSK) race in 1999 imposed a serious threat to wheat production

worldwide due to its virulence against *Sr31* (Singh *et al.*, 2011). Ug99 had been confirmed in Uganda, Kenya, Ethiopia, Sudan, and Yemen. Occurrence of Ug99 in Yemen was a significant confirmation that it was moving toward the important wheat growing areas of the Middle East and Asia. Afterward its presence in Iran was confirmed in March 2008 and supported these predictions. Iranian Ug99 isolates were collected from Borujerd and Hamadan, in northwestern Iran (Nazari *et al.*, 2009). Also, to date it is not known to have spread beyond Iran. Unusual occurrence of stem rust in Pakistan in 2009 imposed some fears of an Ug99 incursion, but phenotyping of the samples and DNA analysis revealed the absence of Ug99 and presence of another important race (Mirza *et al.*, 2010; Singh *et al.*, 2011). Changes in pathogen populations are rapid. To date, 8 pathotypes have been reported in Ug99 lineage which each has a unique combination of virulence and they differ for

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virulence to resistance genes *Sr21*, *Sr24*, *Sr31* and *Sr36* (Jin *et al.*, 2008, 2009; Park *et al.*, 2011; Pretorius *et al.*, 2010, 2012). These pathotypes have been shown to have identical fingerprints using microsatellite markers, suggesting that they have risen from a common ancestor via single-step mutation (Pretorius *et al.*, 2010).

Molecular approaches based on polymerase chain reaction (PCR) have become efficient tools for quick detection and study of the plant pathogens (Edel, 1998; Mutasa *et al.*, 1995; Ward, 1994). The internal transcribed spacer (ITS) regions are useful for targets and have been used for specific detection of numerous plant pathogens (Ge and Guo, 2003; Ghignone *et al.*, 2003; Sholberg *et al.*, 2004; Yang *et al.*, 2003; Zhao *et al.*, 2007). House-keeping genes are considered valuable for studies on population structure and macroevolution; this is in contrast with virulence/avirulence genes which are subjected to strong host selection (Taylor *et al.*, 1999). In recent years, *EF1α* and *β-tub1* genes (Van der Merwe *et al.*, 2007) and *nucLSU rDNA* (Maier *et al.*, 2007) sequences have been used to study phylogeny of Pucciniaceae. Although, *rDNA ITS* regions have proven helpful for phylogenetic studies as well (Alaei *et al.*, 2009; Abbasi *et al.*, 2004; Chatasiri *et al.*, 2006; Kropp *et al.*, 1997; Pei *et al.*, 2005; Smith *et al.*, 2004; Szabo, 2006; Weber *et al.*, 2003; Wood and Crous, 2005; White *et al.*, 1990). There are advantages to use *rDNA ITS* regions in phylogenetic studies including, multi copy nature, inter specific diversity and availability in gene bank (Barnes and Szabo, 2007; Hamelin *et al.*, 1996; Schena *et al.*, 2004).

As study of phylogenetic relationships of Iranian isolates of *Pgt* Ug99 race group with other *Pgt* isolates have not been performed, the object of this study was phylogenetic characterization of *Pgt* TTSSK race in Iran based on molecular markers.

Materials and Methods

Stem rust isolate

An isolate of *Pgt* which was collected from Dasht-e Azadegan (Khuzestan province) was received from the Department of Cereal

Research, Seed and Plant Improvement Institute Karaj, Iran. The isolate was maintained and purified on Morocco cultivar using McIntosh *et al.* (1995) method. Seven-day-old seedlings were inoculated by urediniospores. Seedlings were incubated at 18 °C in a dew chamber in darkness for 14h. Then the inoculated plants were placed on the greenhouse bench at 25 ± 2 °C with a photoperiod of 16h (Jin *et al.*, 2007). 14 days after inoculation single pustule was used for inoculation of healthy susceptible seedlings. Afterward single-postulated isolate was increased on Morocco cultivar. Race identification was done based on differential sets received from CIMMYT and ICARDA (Jin *et al.*, 2008) and designated as TTSSK (Mojerlou *et al.*, 2013). The difference between TTSSK and TTKSK was confirmed by virulence against *Sr36* and avirulence against *Sr17* using Combination VII (*Sr17*), LC/ Kenya Hunter (*Sr17*) and W2691SrTt-1 (*Sr36*) differential lines.

Genomic DNA extraction

Total DNA was extracted according to Roos-Amsaleg *et al.* (2002) method with some modifications. Three DNA samples were extracted from single- postulated urediniospores and were considered as Pgt1, Pgt2 and Pgt3. 1mg of non-germinated urediniospores was ground in a 1.5ml micro tube with 50μl of extraction buffer (100mM Tris-HCl, pH 9.0; 20mM EDTA, pH 8.0; 1.4mM NaCl, 2% CTAB). Then total volume reached to 600μl by adding extraction buffer. The solution was incubated for 2h at 65 °C. Then 600μl phenol: chloroform: isoamyl alcohol (25:24:1, v/v/v) was added to each tube. Phases were separated by 13000 rpm centrifugation. Upper phase was transferred to 1.5ml micro tube and DNA was precipitated with 700μl of cold isopropanol. DNA pellet was dried and re-suspended in 50μl sterilized deionized water (Wang *et al.*, 2009). The extracted DNA was treated with RNase A. Then quality and quantity of the isolated DNA were determined using agarose 1% (w/v) electrophoresis and spectrophotometer (Eppendorf 6131) measurement. DNA samples were adjusted to final concentration of 50 ng/ μl for PCR amplification.

ITS amplification

Three isolates of *Pgt* race TTSSK were used in PCR amplification of ITS region with universal primers ITS1F (5'-CTTGGTCATTAGAGGAAGTAA-3') (Gardes and Bruns, 1993) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). PCR parameters were: an initial denaturation for 5 min at 94 °C, then 40 cycles consisting of denaturation at 94 °C for 1 min, annealing at 46 °C for 1 min and extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. sterilized distilled water was used as negative control. PCR products were electrophoresed in a 1.2% (w/v) agarose gel containing ethidium bromide (0.5µg/ml) in 1X TAE buffer. Approximately 700bp of an ITS1, 5.8S and ITS2 regions were amplified using ITS1F and ITS4 primers.

Sequencing and phylogenetic analysis

PCR products were direct sequenced. Sequencing was performed by the Pishgam Biotech Company (www.pishgambc.com). Blast searching of ITS sequences was done and the sequences were aligned using Mega 6.0 software. Clustering method Maximum Parsimony (MP) was used to estimate the phylogeny with the aid of Mega 6.0. 1000

bootstrap replicates were performed. Consensus tree was produced by Mega 6.0 too.

Results and Discussion

Amplified segment (Fig. 1) for three samples of *Pgt* race TTSSK from Iran were sequenced and aligned together with related sequences obtained from Genbank. Afterward data were analyzed by Mega 6.0 software and a consensus tree was estimated. The estimated consensus tree for ITS region using MP clustering method is shown in Fig. 2.

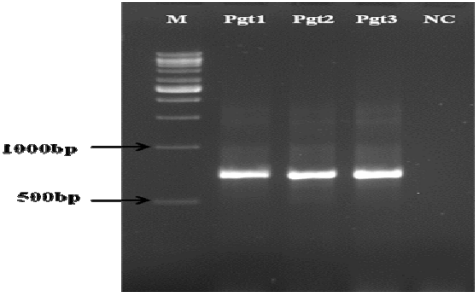


Figure 1 Ethidium bromide- stained agarose gel of Polymerase chain reaction products with universal primers ITS1F and ITS4 from 3 isolates of *Puccinia graminis* f.sp. *tritici* (*Pgt*). NC = Negative control (sterile water) and M = 1Kb DNA ladder (NL-1411Vivantis).

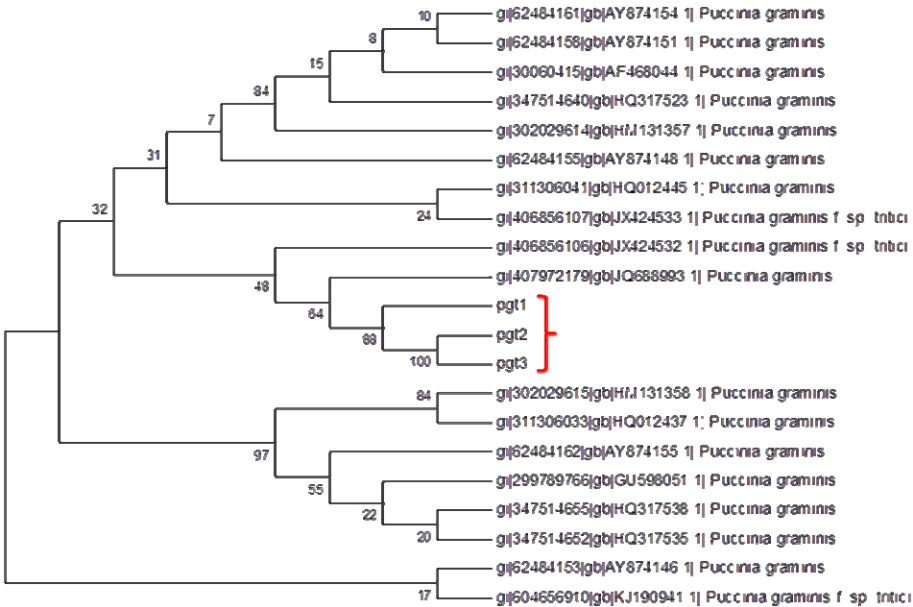


Figure 2 Phylogenetic relationships of a selection of isolates in the *Puccinia graminis* species based on Maximum Parsimony analysis of ITS sequences.

As the three DNA samples were extracted from one *Pgt* isolate, they were grouped together. Samples of Pgt2 and Pgt3 were similar and supported well (bootstrap 100). Interestingly they showed to be slightly different from Pgt1. It suggested that they may be mixed during single postulation process. The phylogenetic analysis showed a monophyletic core group of *P. graminis* divided into two main groups, A and B. Iranian isolate of TTSSK was placed in a clade with *P. graminis* (JQ688993) which was isolated from *Berberis* sp. (Berlin *et al.*, 2012) and one *Pgt* isolate from Tajikistan (JX424532). To conclude whether the surface-level winds were involved in spread of *Pgt* from Iran to Tajikistan, more meteorological and weather satellite information is needed. Nagarajan *et al.* (2013) showed that the spread of Ug99 urediniospores from Africa to Lorestan and Hamadan Province, Iran, was not likely to occur due to surface-level winds and something unusual- a tropical cyclone Gonu- happened in 2007. The cyclone crossed the eastern-most tip of Oman and turned to the north-northwest and made landfall on the Makran coast of Iran. They concluded if Gonu Had enabled the spread of Ug99 -TTKSK from Ethiopia to Iran, then why other *Pgt* virulence types from Ethiopia were not recorded in Iran at that time. Therefore the North Arabian Sea tropical cyclones and western distribution were not involved with the appearance of Ug99 in Iran in 2007.

The results showed that most of the isolates which were collected from grass species hosts including *Poa* sp., *Festuca* sp., *Lolium* sp. and *Avena* sp. were grouped together in clade B and the isolates from *Triticum aestivum*, *Secale cereals*, *Agropyron repens* were grouped together in clade A. The Iranian isolate was placed in clade B. The ITS region sequences have been used for detection, identification and phylogenetic analysis of several plant pathogens including rust fungi and our results agree with those of other researchers (i.e Alaei *et al.*, 2009; Zhao *et al.*, 2007). *P. graminis* is a complex species and several methods have been used to classify the divisions, and *formae*

speciales, subspecies and varieties are representative examples of complexity in this species (Berlin *et al.*, 2012).

Abbasi *et al.* (2004) showed that the species, *Pgt*, is highly complex based on ITS sequence and that morphological and genetic characters by themselves are not useful to clarify evolutionary relationship of samples from different parts of the world. Berlin *et al.* (2012) showed the high genetic diversity within each *formae speciales* of *P. graminis*. Our results showed that more than one conserved genomic region would be informative to identify phylogenetic relationship of Iranian *Pgt* isolates and samples from different parts of the world. This result is corresponding to other researchers (Abbasi *et al.*, 2004; Berlin *et al.*, 2012).

In conclusion, in this study we determined rDNA ITS sequence information from Iranian isolate of *Pgt* race TTSSK which can be a variant of Ug99 race group and it would be useful to clarify phylogenetic relationship of Iranian isolates and other isolates from different parts of the world. As *Pgt* is highly adapted to long distance migration, this information would be helpful to determine the movement route of Ug99 and show the lineage of Iranian isolates. Of course complementary studies with more isolates and more sequence data from other genomic loci are needed.

References

- Abbasi, M., Hedjaroude, G., Scholler, M. and Goodwin, S. B. 2004. Taxonomy of *Puccinia striiformis* s. l. in Iran. *Rustaniha*, 5: 199-224.
- Alaei, H., De Backer, M., Nuytinck, J., Maes, M., Hofte, M. and Heungens, K. 2009. Phylogenetic relationships of *Puccinia horiana* and other rust pathogens of *Chrysanthemum × morifolium* based on rDNA ITS sequence analysis. *Mycological Research*, 113: 668-683.
- Barnes, C. W. and Szabo, L. J. 2007. Detection and identification of four common rust pathogens of cereals and grasses using real

- time polymerase chain reaction. *Phytopathology*, 97: 717-727.
- Berlin, A., Djurle, A., Samils, B. and Yuen, J. 2012. Genetic variation in *Puccinia graminis* collected from oats, rye, and barberry. *Phytopathology*, 102: 1006-1012.
- Chatasiri, S., Kitade, O. and Ono, Y. 2006. Phylogenetic relationships among *Puccinia hemerocallidis*, *P. funkiae*, and *P. patriniae* (Uredinales) inferred from ITS sequence data. *Mycoscience*, 47: 123-129.
- Edel, V. 1998. Polymerase chain reaction in mycology. In: Aroa, D. K., Reddy, C. A., Bridge, P. D. and Elander, R. P. (Eds.), *Application of Polymerase Chain Reaction in Mycology*. CAB Publishing, New York, pp. 1-20.
- Gardes, M. and Bruns, T. D. 1993. ITS primers with enhanced specificity for Basidiomycetes: application to the identification of mycorrhizae and rusts. *Molecular Ecology*, 2: 113-118.
- Ge, Y. Y. and Guo, J. H. 2003. Analysis of ITS sequence and PCR protocol for the detection of *Clavibacter fangii*, the causal agent of wheat seedling wilting disease. *Acta Phytopathologica Sinica*, 33: 198-202.
- Ghignone, S., Tamietti, G. and Girlanda, M. 2003. Development of specific PCR primers for identification and detection of *Rhizopycnis vagum*. *European Journal of Plant Pathology*, 109: 861-870.
- Hamelin, R. C., Berube, P., Gignac, M. and Bourassa, M. 1996. Identification of root rot fungi in nursery seedlings by nested multiplex PCR. *Applied and Environmental Microbiology*, 62: 4026-4031.
- Jin, Y., Singh, R. P., Ward, R. W., Wanyera, R., Kinyua, M. G., Njau, P., Fetch, T. J., Pretorius, Z. A. and Yahyaoui, A. 2007. Characterization of seedling infection types and adult plant infection responses of monogenic *Sr* gene lines to race TTKS of *Puccinia graminis* f. sp. *tritici*. *Plant Disease*, 91: 1096-1099.
- Jin, Y., Szabo, L. J., Pretorius, Z. A., Singh, R. P., Ward, R., and Fetch, T. 2008. Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. *Plant Disease*, 92: 923-926.
- Jin, Y., Szabo, L. J., Rouse, M., Fetch, T., Pretorius, Z. A., Singh, R. P., Wanyera, R. and Njau, P. 2009. Detection of virulence to resistance gene *Sr36* within race TTKS lineage of *Puccinia graminis* f. sp. *tritici*. *Plant Disease*, 93: 367-370.
- Kropp, B. R., Hansen, D. R., Wolf, P. G., Flint, K. M. and Thomson, S. V. 1997. A study on the phylogeny of the dyer's woad rust fungus and other species of *Puccinia* from crucifers. *Phytopathology*, 87: 565-571.
- Leonard, K. J. and Szabo, L. J. 2005. Stem rust of small grains and grasses caused by *Puccinia graminis*. *Molecular Plant Pathology*, 6: 99-111.
- Maier, W., Wingfield, B. D., Mennicken, M. and Wingfield, M. J. 2007. Polyphyly and two emerging lineages in the rust genera *Puccinia* and *Uromyces*. *Mycological Research*, 111: 176-185.
- McIntosh, R. A., Wellings, C. R. and Park, R. F. 1995. *Wheat Rusts: An Atlas of Resistance Genes*. Victoria, Australia: CSIRO, 213 pp.
- Mirza, J. I., Rattu, A., Khanzada, K. A., Ahmad, I. and Fetch, T. 2010. Race analysis of stem rust isolates collected from Pakistan in 2008-09. *Proceedings of BGRI 2010 Technical Workshop*, St Petersburg, Russia, May 30-31, p. 5.
- Mojerlou, Sh., Safaie, N., Abasi-Moghadam, A. and Shams-Bakhsh, M. 2013. Evaluation of some Iranian wheat landraces resistance against stem rust disease at seedling stage in the greenhouse. *Plant Protection Journal*, 35: 69-82 (In Farsi).
- Mutasa, E. S., Chwarszczynska, D. M., Adams, M. J., Ward, E. and Asher, M. J. C. 1995. Development of PCR for the detection of *Polymyxa betae* in sugar beet roots and its application in field studies. *Physiological and Molecular Plant Pathology*, 47: 303-313.
- Nagarajan, S., Kogel, H. J. and Zadoks, J. C. 2013. Influence of the Arabian Sea tropical cyclone and the Western disturbance on the appearance of *Puccinia graminis* f. sp. *tritici*

- virulence Ug99 -TTKSK in Iran in 2007. *Current Science*, 105 (2): 164-166.
- Nazari, K., Mafi, M., Yahyaoui, A., Singh, R. P. and Park, R. F. 2009. Detection of wheat stem rust (*Puccinia graminis* f. sp. *tritici*) race TTKSK (Ug99) in Iran. *Plant Disease*, 93: 317-317.
- Park, R., Fetch, T., Hodson, D., Jin, Y., Nazari, K., Prashar, M. and Pretorius, Z. 2011. International surveillance of wheat rust pathogens: progress and challenges. *Euphytica*, 179: 109-117.
- Pei, M. H., Bayon, C. and Ruiz, C. 2005. Phylogenetic relationships in some *Melampsora* rusts on Salicaceae assessed using rDNA sequence information. *Mycological Research*, 109: 401-409.
- Pretorius, Z. A., Bender, C. M., Visser, B. and Terefe T. 2010. First report of a *Puccinia graminis* f. sp. *tritici* race virulent to the *Sr24* and *Sr31* wheat stem rust resistance genes in South Africa. *Plant Disease*, 94: 1163-1163.
- Pretorius, Z. A., Jin, Y., Bender, C. M., Herselman, L. and Prins, R. 2012. Seedling resistance to stem rust race Ug99 and marker analysis for *Sr2*, *Sr24* and *Sr31* in South African wheat cultivars and lines. *Euphytica*, 186: 15-23.
- Roose-Amsaleg, C., de Vallavieille-Pope, C., Brygoo, Y. and Levis, C., 2002. Characterisation of a length polymorphism in the two intergenic spacers of ribosomal RNA in *Puccinia striiformis* f. sp. *tritici*, the causal agent wheat yellow rust. *Mycological Research*, 106: 918-924.
- Schena, L., Nigro, F., Ippolito, A. and Gallitelli, D. 2004. Real-time quantitative PCR: a new technology to detect and study phytopathogenic and antagonistic fungi. *European Journal of Plant Pathology*, 110: 893-908.
- Sholberg, P. L., O'Gorman, D. T. and Bedford, K. E. 2004. Use of PCR and DNA hybridization for identification of pear powdery mildew caused by *Podosphaera leucotricha*. *Canadian Journal Plant Pathology*, 26: 199-204.
- Singh, R. P., Hodson D. P., Huerta-Espino J., Jin Y., Njau P., Wanyera R., Herrera-Foessel S. A., Bhavani S., Singh D. and Singh P. K. 2006. Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, 1 (54): 1-13.
- Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin, Y., Bhavani, S., Njau, P., Herrera-Foessel, S. A., P. Singh, P., Singh, S. and Govindan, V. 2011. The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. *Annual Review of Phytopathology*, 49: 465-481.
- Smith, J. A., Blanchette, R. A. and Newcombe, G., 2004. Molecular and morphological characterization of the willow rust fungus, *Melampsora epitea*, from arctic and temperate hosts in North America. *Mycologia*, 96: 1330-1338.
- Szabo, L. J. 2006. Deciphering species complexes: *Puccinia andropogonis* and *Puccinia coronata*, examples of differing modes of speciation. *Mycoscience*, 47: 130-136.
- Taylor, J. W., Jacobson, D. J. and Fisher, M. C. 1999. The evolution of asexual fungi: reproduction, speciation and classification. *Annual Review of Phytopathology*, 37: 197-246.
- Van der Merwe, M., Ericson, L., Walker, J., Thrall, P. H. and Burdon, J. J. 2007. Evolutionary relationships among species of *Puccinia* and *Uromyces* (Pucciniaceae, Uredinales) inferred from partial protein coding gene phylogenies. *Mycological Research*, 111: 163-175.
- Wang, Y., Zhu, M., Zhang, R., Yang, H., Wang, Y., Sun, G., Jin, S. and Hsiang, T. 2009. Whole genome amplification of the rust *Puccinia striiformis* f.sp. *tritici* from single spores. *Journal of Microbiological Methods*, 77: 229-234.
- Ward, E. 1994. Use of the polymerase chain reaction for identifying plant pathogens.

- In: Blakeman, J. P. and Williamson, B. C., (Eds.), Ecology of Plant Pathogens. Oxford University Press, Wallingford, UK, pp. 143-160.
- Weber, R. W. S., Webster, J. and Engel, G. 2003. Phylogenetic analysis of *Puccinia distinct* and *P. lagenophorae*, two closely related rust fungi causing epidemics on Asteraceae in Europe. Mycological Research, 107: 15-24.
- White, T. J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenies. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. (Eds.), PCR Protocols: a Guide to Methods and Applications. Academic Press, San Diego, pp. 315-322.
- Wood, A. R. and Crous, P. W. 2005. Morphological and molecular characterization of Endophyllum species on perennial asteraceous plants in South Africa. Mycological Research, 109: 387-400.
- Yang, P. W., Li, J. R., Yang, Q. Z., Zeng, L. and Wang, Q. 2003. Cloning and sequencing the internal transcribed spacer of the ribosomal gene of *Plasmodiophora brassicae* and applying it in detecting the pathogen. Journal of Yunnan Agricultural University, 18: 228-233.
- Zhao, J., Wang, X. J., Chen, C. Q., Huang, L. L., and Kang, Z. S. 2007. A PCR-based assay for detection of *Puccinia striiformis* f. sp. *tritici* in wheat. Plant Disease, 91: 1669-1674.

شناسایی مولکولی نژاد TTSSK قارچ *Puccinia graminis* f. sp. *tritici* از ایران

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چکیده: بیماری زنگ ساقه که توسط عامل *Puccinia graminis* f. sp. *tritici* (*Pgt*) ایجاد می‌شود یکی از بیماری‌های مخرب گندم در سراسر دنیا می‌باشد. عامل بیماری می‌تواند ۳۶۵ گونه مختلف از گیاهان علفی و بیش از ۷۰ گونه زرشک را آلوده نماید. استفاده از توالی ناحیه ITS برای بررسی ارتباط فیلوژنی در سطح گونه و زیرگونه مناسب می‌باشد. در این پژوهش از یک جدایه *Pgt* که از ایران جمع‌آوری و به‌عنوان TTSSK نام‌گذاری شده بود، استفاده شد. سه نمونه از جدایه قارج مورد ارزیابی قرار گرفت. ناحیه ITS نمونه‌ها تکثیر و تعیین توالی شد. درخت توافقی به‌روش ماکزیمم پارسیمونی و با استفاده از نرم‌افزار Mega 6.0 رسم شد. جدایه ایرانی TTSSK در کنار جدایه جدا شده از زرشک و جدایه تاجیکستان قرار گرفت. نتایج نشان داد که بیش از یک ناحیه حفاظت‌شده ژنومی برای شناسایی رابطه فیلوژنتیکی جدایه‌های ایرانی *Pgt* با جدایه‌های سایر مناطق دنیا مورد نیاز می‌باشد. مطالعات تکمیلی به‌کمک جدایه‌های بیشتر و توالی سایر نواحی حفاظت‌شده ژنوم در حال انجام می‌باشد.

واژگان کلیدی: ITS، ماکزیمم پارسیمونی، رابطه فیلوژنتیکی، زنگ ساقه، گروه نژادی Ug99