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GENETIC VARIABILITY OF *Rhizoctonia* spp. ISOLATED FROM DIFFERENT HOSTS AND LOCATIONS

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Abstract: *Rhizoctonia* species is well known as a destructive fungal pathogen in various agro-ecosystems globally. The current study attempted to investigate the genetic diversity among various anastomosis groups of *Rhizoctonia* species obtained from different locations and crops. Two types of markers were used to detect the genetic variability among isolates. The results showed that all the molecular markers were able to show reasonable polymorphisms. The clustering tree and PCA plot analysis supported the separation based on taxonomic groups more than the separation according to geographical origin and host. This indicates that there are other important factors that contribute to the genetic variation among *Rhizoctonia* isolates. The knowledge gathered in this study would be useful for developing crops that are resistant to *Rhizoctonia* diseases. This will assist in planning for the right crop rotation and proper disease management programs.

Keywords: *Rhizoctonia*, genetic diversity, Anastomosis groups.

INTRODUCTION

Rhizoctonia spp. are prevalent imperfect soilborne fungi in arable and non-arable lands that led to significant economic losses in crops. In Malaysia, *Rhizoctonia* species has been reported as a fungal pathogen on many crops including rice, corn, chilly, bean and durian. However, studies on characterization of *Rhizoctonia* spp. based on anastomosis groups and genetic variations are lacking. Molecular techniques were found to be more convenient and straightforward to place unknown isolates of *Rhizoctonia* spp. in the right taxonomic anastomosis group. Also, it provides an informative explanation of the genetic variations among and between different populations of *Rhizoctonia* species. Different types of molecular markers such as Randomly Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeat (ISSR) have been used to detect genetic variability. Guleria et al. (2007) studied the genetic variation of *R. solani* isolates that caused sheath blight and found that RAPD markers were more superior in genetic variation detection than ISSR markers. Other markers such as simple sequence repeats (SSR) are very important in genetic studies and can be used to explain the mechanism of sexual reproduction and asexual stage as well as geographical distribution (Arakawa and Inagaki, 2014).

METHODS

Sample collection

Infected crops with *Rhizoctonia* species were gathered from different locations in Selangor (Serdang UPM, Tanjung Karang, Kuala Selangor, Puchong and Sepang), Perak (Telong Intan), Pahang (Cameron Highland), Melaka, Kedah and Kelantan as well as different crops (rice, corn, chilly, spinach and chrysanthemum) in Peninsular Malaysia.

Fungal pathogen isolation

Infected plant tissues were washed with tap water to remove the soil residues. Plant tissues were cut to small pieces of 10 mm and surface disinfected with 3% sodium hypochlorite solution (NaOCl) for three minutes and then rinsed with sterilized distilled water (SDW). Hereafter, infected tissues were blotted on sterilized filter paper and transferred to water agar plates then incubated at 28±1 ºC for three days. The fungal pathogen was sub-cultured on potato dextrose agar medium and kept in slant agar at 4 ºC.

Molecular study

The genomic DNA was isolated using Talbot (2001) method. Thirty-seven isolates of *Rhizoctonia* spp. were subjected to analysis using two types of markers, RAPD and iPBS which are reported to be highly polymorphic. PCR reactions for all markers were carried out using 2X Master Mix EconoTaq® PLUS GREEN (Lucigen, Middleton, USA) in a total volume 30 µl [15 µl ready master-mix, 0.6 µl (0.4µM) primer, 1 µl DNA template (25 ng) and made up to 30 µl with nuclease free water]. The thermal cycler PCR profile program for RAPD included initial denaturation at 95 ºC for 4 min, followed by 40 cycles: denaturation at 95 ºC for 45 secs, annealing at 36 ºC (OPA13), 45 ºC (OPE-6) 43 ºC (2080 iPBS) and 35.7 ºC (2249 iPBS) for 45secs and extension at 72 ºC for 1 min. A final extension at 72 ºC for 10 min was added.

All amplified PCR products used as markers were resolved by 1.5% (w/v) agarose in 1X TAE buffer at 85V for 60 minutes. The agarose gel was stained using Fluoro Safe DNA Stain (4-6 µl per 100 ml) and then visualized by UV transilluminator. All obtained marker bands were repeated twice to check for the reproducibility of the results.

DNA fingerprinting analysis of the different markers was done by scoring using UVIDoc software version 99.02 where each band that was present was scored as 1 and 0 for the absence of band for the specific molecular size shown on the photo of the agarose gel. The scoring was converted to a binary matrix. Numerical taxonomy and multivariate analysis system (NTSYS version 2.1; Exeter Biological Software, Setauket, NY, USA) was used to analyze the data. The input of data was formatted based on the software manual. The coefficient similarity was calculated by Jaccard's and used to construct the dendrogram tree by UPGMA (Unweighted Pair Group Method with Arithmetic Mean) algorithm. PCA (Principal Coordinate Analysis) was constructed using Jaccard's similarity matrix with decenter and Eigen in NTSYS-PC. Molecular variance (AMOVA) was estimated using GenAlEx software version 6.50 (Peakall and Smouse 2012).

RESULTS AND DISCUSSION

In total, 37 isolates of *Rhizoctonia* spp. were subjected to the genetic variation analysis using RAPD (OPE-6 and OPA13) and iPBS (2080 iPBS and 2249 iPBS). RAPD markers showed polymorphic loci (24-43 loci)

while iPBS showed 28-34 polymorphic loci. Polymorphism percentage ranged between 91-92 and 87-100 for RAPD and iPBS, respectively. The analysis of molecular variance (AMOVA) revealed that the differences in variability within the population were 77% while the variation differences between the populations were around 23% (Figure 1).

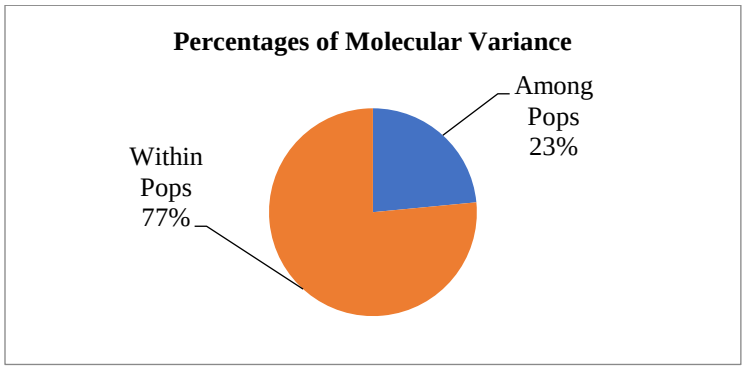

Figure 1. Analysis of Molecular Variance (AMOVA) between and within Populations of 37 Strains of *Rhizoctonia* species. Pops abbreviation for populations.

The data obtained from the different markers were combined and analyzed with UPMGA algorithm and Jaccard clustering to construct the dendrogram tree (Figure 2). Six clusters were obtained according to UPMGA algorithm and Jaccard clustering. Three clusters belonged to *R. solani* AG-1 IA isolated form rice and corn while cluster four belonged to AG-1 ID and AG-A and cluster five and six belonged to AG-Fa and AG-4 HG-I, respectively. The principle of coordinate analysis separated the isolates of *Rhizoctonia* spp. to several groups. However, the separation was according to taxonomic level of anastomosis groups rather than separation according to the locations and host. Dubey et al. (2012) noted that *R. solani* isolated from various pulse hosts were clustered into different clusters based on AG concept. There was no association between the diversity of *R. solani* AG-1-IA strains and their pathogenicity and geographical locations (Khodayari et al., 2009; Dubey et al., 2012). Pourmahdi and Parissa, (2014) implied that genetic variation could be attributed to geographical locations and some other factors such as crop rotation, mutations, gene flow, genetic drift and other possible factors which could be responsible for the variation among *Rhizoctonia* species.

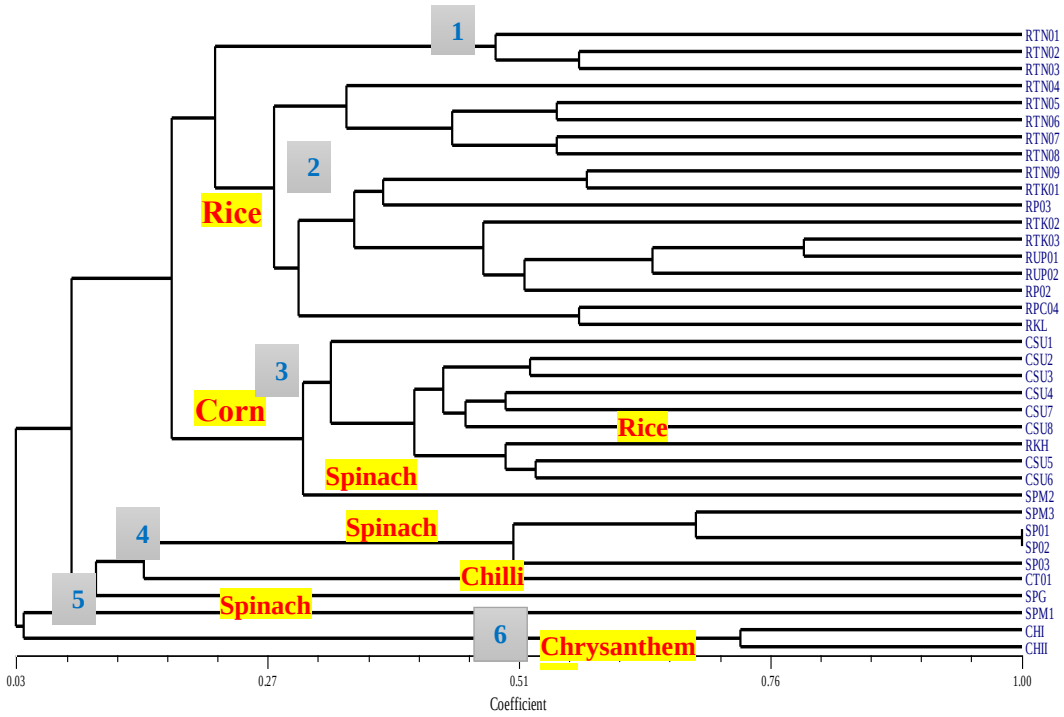

Figure 2: Dendrogram Constructed from Combined Data of Different Markers (RAPD and iPBS) of 37 Isolates of *Rhizoctonia* spp.

CONCLUSIONS

Based on the current and previous studies, the genus of *Rhizoctonia* is one of the most complex and diverse filamentous fungi which requires more in depth studies in order to obtain a comprehensive knowledge about its genetic variability and population structure. In this study, we found that there was no correlation between the genetic diversity, geographical origin and host which implied that there are other factors such as gene flow and genetic drift that could play significant roles in contributing to the genetic variation among and between the fungal pathogen isolates in this study. The future direction of research should aim to study the genetic diversity of *Rhizoctonia* in-depth through screening the different isolates using other type of markers especially single nucleotide polymorphism which is more abundant and widespread throughout the genome.

REFERENCES

Arakawa, M., & Inagaki, K. (2014). Molecular markers for genotyping anastomosis groups and understanding the population biology of Rhizoctonia species. Journal of general plant pathology, 80(5), 401-407.

Dubey, S. C., Tripathi, A., & Upadhyay, B. K. (2012). Molecular diversity analysis of Rhizoctonia solani isolates infecting various pulse crops in different agro-ecological regions of India. Folia microbiologica, 57(6), 513-524.

Guleria, S., Aggarwal, R., Thind, T. S., & Sharma, T. R. (2007). Morphological and pathological variability in rice isolates of Rhizoctonia solani and molecular analysis of their genetic variability. Journal of Phytopathology, 155(11-12), 654-661.

Khodayari, M,, Safaie, N., & Shamsbakhsh., M. (2009). Genetic diversity of Iranian AG1-IA isolates of Rhizoctonia solani, the cause of rice sheath blight, using morphological and molecular markers. Journal of Phytopathol 157:708–714.

Peakall, R., & Smouse, P.E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28, 2537-2539.

Pourmahdi, A., and Taheri, P. (2014). Genetic Diversity of Thanatephorus cucumeris Infecting Tomato in Iran. Phytopathology, 163 : 19-32.