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Genetic differentiation within the *Puccinia polysora* population occured on inbred lines of maize in Thailand

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Corn rust disease caused by *Puccinia polysora* (southern rust), is the air born pathogen that is widespread within and among corn field locations in Thailand The objectives of study were to estimate genetic differentiation of *P. polysora* populations on Kasetsart inbred line (Ki1-Ki52) which planted in different geographical localities of Thailand and to identify the genotype of *P. polysora* using ISSR marker. The corn rust disease severity was evaluated on the 52 varieties of inbred lines (Ki1-Ki52) planted in the province Lampang, Nakhon ratchasima, Songkhla and Kanchanaburi of Thailand. The results showed that rust disease occurred on numerous varieties in Lampang, Nakhon ratchasima and Songkhla. While, the rust disease was not found in Lampang. The Ki36 and Ki38 varieties were resistant to rust disease in all evaluated locations, while the rust symptom with highly disease severity was found on Ki25 and Ki26 in Nakhon ratchasima and Songkhla provinces. Genetic differentiation of *P. polysora* populations on Kasetsart inbred lines (Ki1-Ki52) was analyzed using ISSR markers. The results showed that there were high genetically differentiated within *P. polysora* populations (G_{ST} = 0.1348). Result showed five genotypes among 39 genotypes which found more than one province such as Gen1 Gen14 Gen20 Gen27 and Gen37. Two of them, Gen20 and Gen27 were observed in three provinces. The results supported that there was the migration of urediospores among these provinces.

Keywords: corn rust, *Puccinia polysora*, southern rust, disease resistance

Introduction

Maize or corn (*Zea may* Linn.) is an economic crop in Thailand. Corn rust disease caused by *Puccinia polysora* (southern rust), is considered as major pathogens in corn production of Thailand. *Puccinia polysora* is the air born pathogen widespread within and among corn field locations. (Jintana *et al*., 2011) In the present, many high yields and resistance varieties were

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developed and introduced to the farmers in order to increase the production. The corn improvement program was conducted since 1959 by Corn Breeding of Kasetsart University (KU). According to the KU open-pollinated varieties results, there were 4 varieties officially released namely Suwan 1 (1975), Suwan 2 (1979), Suwan 3 (1987) and Suwan 5 (1993). Afterward, the KU inbred lines (Ki) were also officially released to the public and private sectors. They were as the following: Ki 1 to Ki 19 in 1992; Ki 21 and Ki 22 in 1985; Ki 23 to Ki 30 in 1987; Ki 31 to Ki 44 in 1991 and Ki 45 in 1995 (Aekatasanawan *et.al.*, 1996) The genetic structure of plant parasite is very important data for the plant breeding program to selection the disease resistant variety. The authors have been studied on genetic variation, differentiation or diversity of various rust fungi using molecular makers such as SSR, ISSR and AFLP. (Bruns *et al*., 1990; Lee and Taylor, 1990; Lorsuwan *et al*., 1984; Pfunder *et.al*., 2001; Rohlf, 1993; Roy *et al*., 1998; White, 2000; Manuela *et al*., 2005.).

Inter simple sequence repeat (ISSR) marker is a simple and rapid technique, requires no sequence information and using a single primer for detection and random amplification like RAPD marker. Only small amounts of DNA template are required and the results are clearly scorable demonstrated. (Ratanacherdchai *et al.*, 2010; Jintana *et al*., 2011) The objective of study were to estimate genetically differentiation of *P. polysora* populations on Kasetsart inbred line (Ki1-Ki52) that are grown in different geographical areas and number of genotype of *P. polysora* populations on Ki1-Ki52 varieties.

Materials and methods

Pathogenicity test

The Kasetsart inbred lines (Ki1-Ki52) were planted in the four different geographical provinces of Thailand such as Lampang (North), Nakhon ratchasima (Norht east), Songkhla (South) and Kanchanaburi (West). The experiment was conducted using Randomized Complete Block Design (RCBD) with three replications in each treatment. The rust disease was occurred by infection of natural inoculums after plantation. The rust symptom and disease virulence were recorded at 45, 60, 70 and 100 days after plantation using the rating according to Reid and Zhu (2005) as follows 1 = no symptom, 2 = < 1%, 3= 1-10%, 4= 11-25% of leaves symptomatic, 5= > 50% upper leaves symptomatic, 6 = lower leaves are dead, > 50% of the center leaves, < 25% of upper leaves are symptomatic, and 7= plant is dead.

DNA extraction

DNA was extracted from single uredium (100-200 urediospores). Urediospores were crushed between two sterile glass slides and suspended in 20 µl extraction buffer containing 10 mM Tris-HCl, pH 8.3, 1.5 mM MgCl2, 50 mM KCl, 0.01% SDS, incubated at 37ºC for 60 min, and then at 95 ºC for 10 min (Suyama *et al.*, 1996; Virtudazo *et al.*, 2001).

PCR amplification of ISSR region

DNA specimens were amplified using 20 µl PCR reaction each containing 5 µl of DNA, 10 pmole of single primer, 2.5 units of Taq DNA polymerase and the supplied dNTP mixture (containing 2 mM of each dNTP) and Ex Taq reaction buffer (containing 2 mM Mg2+). PCR was carried out using T professional Standard Gradient (Biometra)under the following condition: 95°C for 5 min, then 30 cycles of 95°C for 1 min, 54°C for 1 min, and 72°C for 1 min, and final step of 72°C for 10 min. The PCR amplification of the ISSR regions were amplified using five primers as following $(AGG)_5$, $GAG(TCG)_5$ and $(GTG)_5$. After amplification, 3 µl of the reaction product was electrophoresed on 1% (w/v) agarose gels containing 0.1µl /ml GelStar (Nucleic Acid Gel Satin, 10000X concentrate in DMSO) in TBE buffer (40 mM Tris, 20 mM sodium acetate, 1 mM EDTA, pH 8.0).

Data collection and analysis

All bands of each primer were manually recorded polymorphic band as binary data by 1 (present) or 0 (absent). The data was analyzed for similarity coefficient and clustering using UPGMA (Nei, 1972) in POPGEN program and GenALEX program for analysis of genetic variabirity of *P. polysora* population. Genetic differentiation via the AMOVA with 999 permutations of the data set was calculated for the ISSR genotypes. An analogous measure developed for binary data, Φ_{PT} was used to calculate differentiation of the genotype in the ISSR groups. Moreover, the band pattern or genotype was evaluated.

Results and discussions

Pathogenicity test

The corn rust disease severity was evaluated on the Kasetsart inbred line (varieties Ki1-Ki52) planted in different geographical provinces of Thailand such as: Lampang, Nakhon ratchasima, Songkhla and Kanchanaburi. The

results showed that the rust disease occurred on various varieties planted in Lampang, Nakhon ratchasima and Songkhla but not occurred on all varieties planted in Kanchanaburi. The Ki36 and Ki38 varieties showed that the rust disease free in all evaluated locations, while the rust symptom with highly disease severity was found on Ki25 and Ki26 varieties in Nakhon ratchasima and Songkhla. However, the disease severity level was different on varieties in each location. The 106 specimens of rust diseases were collected and observed on morphological characteristics under the microscope. Morphological observation on the characteristics of rust pustules on the infected leaves demonstrated the diversity on the symptomatology including colors, shapes and the distribution of the pustules. (Fig. 1) The shape of uredium on leaf was circular to elongate. Most of urediospores occurs with paraphysis in uredium. (Fig. 2) According to the previous report, Jintana *et al*. (2011) observed the rust disease in different locations of Thailand, the results showed that morphological study on the characteristics of rust pustules on the infected leaves and stems demonstrated the diversity on the symptomatology including colors, shapes and the distribution of the pustules.

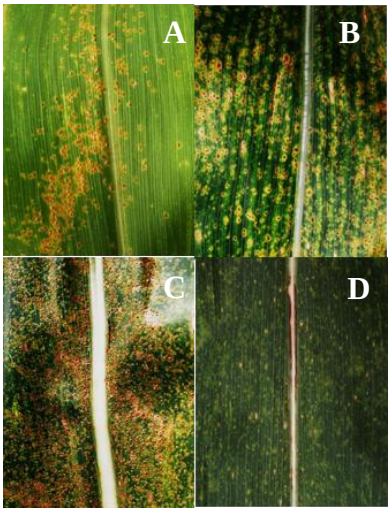

Fig. 1. Rust symptoms caused by *Puccinia polysora* on Kasetsart inbred lines (varieties Ki1-Ki52) planted in different geographical provinces in Thailand; A: Lampang B: Nakorn Ratchasima C: Songkhla and D: Kanchanaburi.

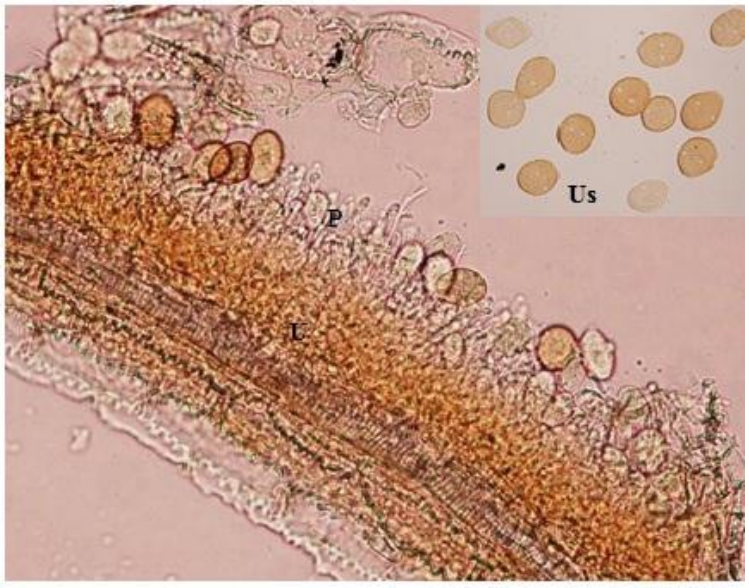

Fig. 2. Morphological characters of uredium (U), paraphysis (P) and urediospore (Us) of *Puccinia polysora* observed under the microscope (400x).

PCR amplification of ISSR region

Genetic differentiation among *P. polysora* populations (31 isolates) on Kasetsart inbred lines (Ki1-Ki52) was analyzed using ISSR marker with three primers, including $(AGG)_5$, $GAG(TCG)_5$ and $(GTG)_5$ primers. The results showed that there were 28 polymorphic bands (fig 3). These binary datas were analyzed using UPGMA method and Gen Alex in POPGEN program, there was highly genetic different within population (92%), while there was lower genetic different among population (8%) by support with Gst value (0.1348) (Fig.4). The population of *P. polysora* in Nakhonratchasima is closely related to the population in Lampang support with Φ_{PT} =0.081 (P>0.05). However, PCA (Principle Coordinate Analysis) based analyzed shows that there were the distribution of samples around the X- and Y-axis (Fig. 5). This result indicated that the distribution of Lampang Songkhla and Nakhon ratchasima populations was correlated to the genotypic pattern. The genotypic identification showed that there were thirty-nine genotypes occurred on the Kasetsart inbred line (Ki). There were 10 genotypes, 27 genotypes and 8 genotypes occurred on Ki varieties planted in Lampang, Nakhon ratchasima and Songkhla, respectively.

However, there were five genotypes that found in more than one province such as Gen1 Gen14 Gen20 Gen27 and Gen37. However, there are two genotypes that occurred in three provinces as Gen20 and Gen27. (Fig. 6) The results supported that there was the migration of urediospores among these

provinces. Puccinia polysora isolates from Lampang and Nakhon ratchasima were closely related based on ISSR genotypes. While, the rust isolates from Songkhla were genetically distinct from the rust isolates from Lampang and Nakhon ratchasima. However, the observation of ISSR pattern showed that there were five genotypes and two genotypes patterns among 39 genotypes found in two provinces and three provinces, respectively. The results indicated that urediospores of *P. polysora* are capable of movement within and between provinces. According to the dissertation of Pattama (2012), the genetic differentiation among populations of P. polysora in Chaiyaphom, Lopburi, Saraburi, Tak, Phetchabun, Chaing Mai, Songkhla and Nakhon Ratchasima were observed and analyzed. The results showed that the genetic differentiation occurred among populations (Gst=0.5103). However, the population in the same geographical area, such as Tak and Phetchabun were more closely related genetically than another populations based on Nei's genetic distance, indicated that the movement of the rust fungi between these areas. The data analyzed in the present study using UPGMA method and Gen Alex in POPGEN program, there was highly genetic different within population (92%), while there was lower genetic different among population (8%) by support with Gst value (0.1348). These results suggested that genetic diversity and differentiation occurred within the population of *P. polysora.* DNA fingerprints detected the same genotypes in different areas suggested that the fungus probary moves regionally and between plantations. The many genes move together as a block in asexual clones which it was called genotype flow.

Genotype flow then refers to the movement of entire genotypes between distinct populations. Genotype flow occurs only for organisms that have a significant asexual component to their life cycle. (www.apsnet.org.) In this case, *P. polysora* produces the repeating spore as call as urediospores, to distribute and infect on maize. So, the entire set of alleles in the clone was introduced into a new population. If this clone had a high degree of fitness, it can be established in the new location. The end of genotype flow was to make population became genetically similar.

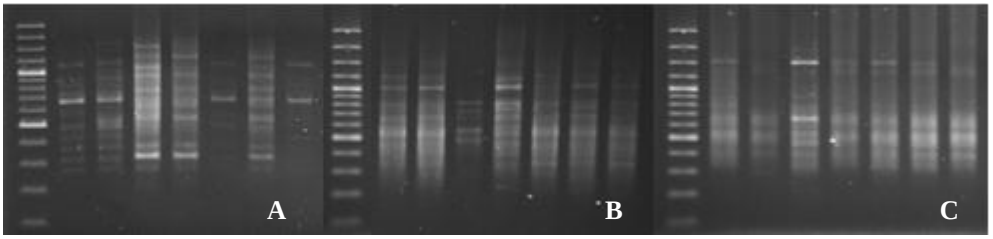

Fig. 3. DNA fingerprint of *Puccinia polysora* by ISSR markers using $(GTG)_5$ (A) $GAG(TCG)_5$ (B) $(AGG)_5$ (C). The first lane in each gel is 100 bp plus DNA Ladder (Fermentas).

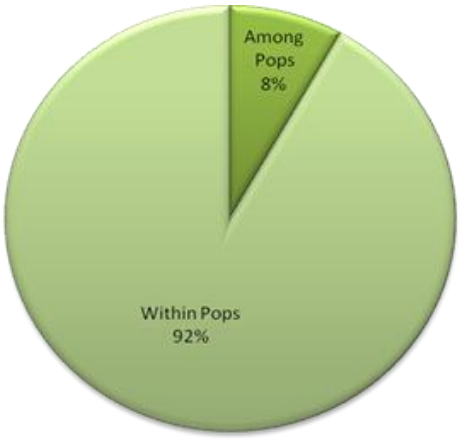

Fig. 4. Molecular variance percentage (%) within and among populations of *Puccinia polysora* based on genetic distance of AMOVA, Φ_{PT} = 0.081 (P>0.05)

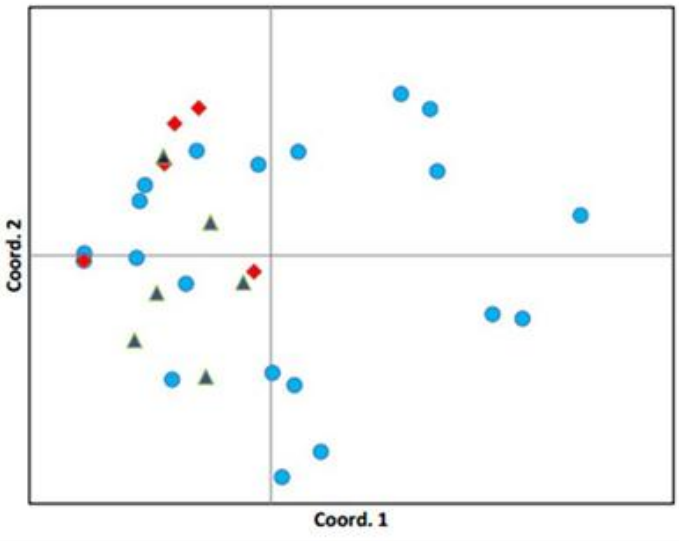

Fig. 5. Principle coordinate analysis plot of inter simple sequence repeat (ISSR) of 31 isolates of *Puccinia polysora* from Lampang (◆) Songkhla (●) and Nakhon ratchasima (▲) based on genetic distance between genotypes.

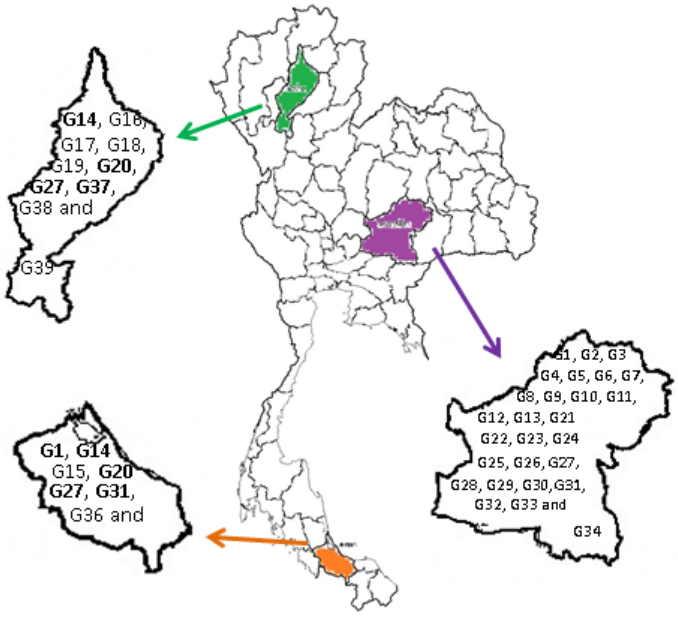

Fig. 6. Genotype diversity of *Puccinia polysora* isolated from three provices of Thailand; Lampang (North), Nakhon ratchasima (East) and Songkhla (South). Genotypes were found in more than one provinces indicated as bold letter.

Summary

The genetic differentiation of *P. polysora* populations on Kasetsart inbred lines (Ki1-Ki52) were highly genetic different within population while there was lower genetic different among populations. Moreover, the genotypic identification showed that genotypic patterns were observed in more than one province. The results indicated that urediospores of *P. polysora* are capable of movement among provinces.

Ackwnoleagement

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