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Genetic diversity among *Fusarium* species associated with sorghum stalk rot in Southern Ethiopia

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Fusaria are very diverse and destructive pathogens affecting different crops. However, their identity and diversity are unresolved in countries like Ethiopia, where various crop species are grown under differing environmental conditions. The objectives of this paper were to identify *Fusarium* spp. associated with sorghum stalk rot in Southern Ethiopia, and elucidate the genetic diversity within and between the species. For this purpose, Fusaria associated with sorghum from two locations in Southern Ethiopia were isolated. Sequencing of the elongation factor 1-alpha gene (EF-1α) was used for species identification. In addition, AFLP analysis was employed for further diversity studies within and between the *Fusarium* spp. Sequence analyses revealed the presence of two *Fusarium* spp. The first was identified as *Fusarium andiyazi*, while the identity of the second remains to be solved. AFLP analysis clustered the isolates into two major groups. The Dice similarity coefficients ranged from 0.39 to 0.91 for isolates of *F. andiyazi* while isolates within the new *Fusarium* spp. had a Dice similarity coefficient varying between 0.69 and 0.96. Cluster analysis and principal coordinate analysis clearly indicated a genetic separation between the two species. Both groups were pathogenic to mature sorghum plants following a toothpick inoculation test. More researches are required to identify the new species and elucidate the pathogenicity of the isolates.

Key words: EF-1α, *Fusarium andiyazi*, genetic similarity, sequence analysis, *Sorghum bicolor*.

INTRODUCTION

Sorghum (*Sorghum bicolor*, (L.) Moench) is the fifth most important cereal accounting for more than 65 million tons of annual production on over 45 million ha of land worldwide (FAO, 2017). The bulk of sorghum is produced in less developed nations (Berenji and Dahlberg, 2004), however, USA is the leading producer with more than 12 million tons of production (FAO, 2017). In Ethiopia, the crop is grown on more than 2 million ha of land making it one of the three most important crops both in terms of area coverage and total production (5 million tons) (CSA, 2018).

Sorghum is grown for its various purposes in different parts of the world. It serves as a major source of food and is also used as feed source for livestock especially in developed nations. In addition, sorghum is used as raw material for industries and for the production of bio-fuel. Despite its versatile use and ability to withstand adverse environmental conditions including moisture stress and...
high temperatures, sorghum production is hampered by various biotic stresses among which diseases caused by different pathogens are one (Thakur and Mathur, 2000; Chala et al., 2011; 2012; Eshte et al., 2015). Fusarium species that cause stalk rot, ear rots and grain mold are among the major pathogens that infect sorghum (Frederiksen and Odvody, 2000; Leslie et al., 2005). The fungus Fusarium belongs to the most harmful pathogens of cultivated crops all over the world (Antonia, 1995; Ramdial et al., 2017).

Once they occur in the field or storage, Fusarium spp. are known to cause significant qualitative and quantitative yield reduction (Parry et al., 1995; Brandfass and Karlovsky, 2008). In addition, they produce mycotoxins that pose serious health risks to humans and animals that feed on contaminated grains (D’Mello et al., 1999; Pestka and Smolinsky, 2005; Antonissen et al., 2014; Wu et al., 2014 and Duan et al., 2016). Despite continued efforts to manage diseases caused by Fusaria, they still pose serious threats to grain producers across the world (Brandfass and Karlovsky, 2008). Since the early reports by Wollenweber and Reinking (1935), lots of researches have been conducted on the taxonomy/genetic diversity of the genus Fusarium (Summerell et al., 2011; O’Donnell et al., 2015; Laurence et al., 2016; Moussa et al., 2017 and Valente et al., 2017). However, research on Fusarium spp. from sorghum has been given only peripheral importance (Leslie et al., 2005). The only exceptions, in this regard, are earlier reports by Claffin (2000) and Leslie (2000; 2002), which identified more than 10 Fusarium species from sorghum, with many of them known to infect the stalk and grain. On the other hand, the identity and diversity of Fusarium species inflicting sorghum in Africa, particularly in Ethiopia remains unresolved. In Ethiopia, there are limited reports (Ayalew, 2002; Ayalew et al., 2006; Chala et al., 2014; Taye et al., 2016; 2018) on Fusarium spp. and associated mycotoxins from sorghum even though the country is one of the primary centers of origin and diversity for the crop. The objectives of this work were: i) to identify Fusarium spp. associated with sorghum stalk rot in Southern Ethiopia; and ii) to elucidate the genetic diversity within and between the species.

MATERIALS AND METHODS

Isolate collection

Twenty sorghum stalks with visible rotting were randomly collected from sorghum fields in Southern Ethiopia during a routine field survey. The stalk samples were stored in paper bags at room temperature until isolation. Geographic description of the locations is given in Table 1.

Isolation, identification and storage of the isolates

Infected stalks were cut into pieces, surface sterilized using 0.5% sodium hypochlorite (NaOCl) solution for 90 s, and rinsed three times in sterile, distilled water. The cut and surface-sterilized stalks were placed on potato dextrose agar (PDA) and incubated at 25°C under continuous fluorescent light for 10 days. After 10 days of incubation, sporulation was observed in the PDA plates. Isolates were identified as Fusaria based on pigmentation and conidial morphology. On these bases, a total of 37 single spore isolates were transferred to new PDA plates. The isolates were grouped into two groups based on pigmentation and form of conidia. Pure cultures were maintained on potato dextrose agar (PDA) amended with 50 ppm of streptomycin, and stored at 4°C as stock cultures.

Molecular characterization

DNA extraction

Approximately 100 mg of fresh mycelium per isolate was crushed in liquid nitrogen using mortar and pestle. Fine powdered mycelium was transferred to a 2 ml microcentrifuge tube and genomic DNA was extracted using the DNAeasy Plant Mini Kit (Qiagen Inc., Valencia, CA) following the manufacturer’s instructions. The quality of the extracted DNA was controlled on 0.8% agarose gels and the DNA was stored at -20°C.

Species identification

Elongation factor 1-alpha genes of seven isolates, randomly selected from the two morphological groups, were partially sequenced using the EF-1α primers that is, EF-728F: 5’-CATCGAGAAGTTCGAGAAGG-3’ and EF-986R: 5’-TACTTGAAGGAAACCTTACC-3’ (Carbone and Kohn, 1999). The resulting sequences were BLAST searched with the NCBI nucleotide database for molecular species identification.

AFLP analysis

AFLP analysis was conducted following the method developed by Vos et al. (1995) with modifications that include the use of fluorescent labeled primers instead of radioactive labeled isotopes. Six combinations of MseI and EcoRI primers were used for selective amplification (Table 2). The primers differ by two selective nucleotides at their 3’ ends and the EcoRI primers were labeled with the fluorescent dye FAM (6-carboxyfluorescein). The selective amplification reaction mix contained 1.6 µl dNTP (2.5 mM), 2 µl of 10× polymerase chain reaction (PCR) buffer, 0.08 µl of Taq DNA polymerase (5 U/µl), and 5 µl MseI (6 ng/µl) and 1 µl EcoRI (1 pmol) primers to which 5 µl of 10 fold diluted preamplification PCR product was added as a template. The PCR amplification conditions were as follows: 1 cycle of 94°C for 30 s, 65°C for 30 s and 72°C for 60 s; 12 cycles where the annealing temperature was lowered by 0.7°C for each cycle; 23 cycles at 94°C for 30 s, 58°C for 30 s and 72°C for 60 s; finally 72°C for 7 min. The accuracy of the analysis was checked by running a randomly selected sample in duplicates.

Data scoring and analysis

Amplification products were separated in an ABI3730 DNA analyzer (Applied Biosystems Inc., Foster City, California) following the manufacturer’s protocol and using GeneScan-1200 LIZ size standard (Applied Biosystems). The presence (1) and absence (0) of peaks were scored using GeneMapper software version 4.0 (Applied Biosystems Inc., Foster City, California), checked manually, and only clear and unambiguous peaks with fluorescence greater than or equal to 100 arbitrary units were entered into a binary data matrix for further analysis. The binary matrices were
then used to calculate genetic similarities between the isolates based on the Dice similarity coefficient (Dice, 1945), and the unweighted pair-group method with arithmetic average (UPGMA) was used to construct a genetic similarity tree with the help of the NTSYS-pc software, version 2.0 (Exeter Biological Software, Setauket, NY). To further elucidate the genetic relationship among the tested isolates, principal coordinate analysis was conducted using the software GenAlEx6 (Peakall and Smouse, 2006).

**In-vitro growth rate of Fusarium isolates**

To study the phenotypic characters (growth rate and colony morphology) of the *Fusarium* isolates, 5 mm portions of the 38 single spore isolates were transferred from the stock cultures and cultivated on PDA at 25°C in the dark. After five days of incubation, 3 mm mycelia plugs were taken from the actively growing edges of each isolate, transferred to the centre of four replicate PDA plates, and incubated in the dark at 25±2 and 30±2°C. For each isolate, radial growth was recorded at 24 h intervals for 7 days.

### RESULTS

**Species identification and characterization**

**Sequence analysis**

Based on sequencing of the elongation factor 1-alpha gene (EF-1α) of isolates, the *Fusaria* were categorized into two groups. The first group of isolates were identified as *F. andiyazi*. The sequence of the second group of isolates did not match with the sequence of *Fusarium* species deposited in NCBI, and hence their identity remains to be resolved.

**Morphological and cultural characterization**

*F. andiyazi* isolates produced both macro- and microconidia and had a white mycelium that become some what pale through time. Isolates belonging to the unidentified *Fusarium* species also produced both micro- and macro- conidia on PDA while their mycelium consistently appeared white with a mass of orange colored spores. Isolates of similar morphological appearance were also obtained from sorghum and finger millet grains collected from different locations in Ethiopia (data not shown).

Isolates belonging to the two species also varied in terms of radial growth rate. The growth rate of isolates belonging to *F. andiyazi* ranged from 8 to 14 mm/day and 8 to 12 mm/day at 25±2 and 30±2°C, respectively. On the other hand, isolates belonging to the new species grew considerably faster (10-17 mm/day) at 25°C than at 30°C (7-12 mm/day). When five isolates representing *F. andiyazi* and 10 isolates from the new *Fusarium* species were used to inoculate the stalks of mature sorghum plants using the toothpick inoculation method (Cumagun et al., 2009), all of them produced typical lesions that were absent in the control plants. This suggested the pathogenicity of both species to sorghum and proved that they were responsible for the stalk rot of sorghum.

**AFLP analysis**

AFLP analysis of 38 isolates clustered them into two major groups (Figure 1). The first major group consists of 16 isolates identified as *F. andiyazi* by sequence analyses. Dice similarity coefficient for isolates belonging to this major group varied from 0.39 to 0.91 (Table 3). Results differentiated isolates of *F. andiyazi* into two subgroups. The first sub-group consists of 7 isolates while the second sub-group is made of the remaining 9 isolates. The second major group consists of 22 isolates belonging to the unidentified *Fusarium* species and hence named as *Fusarium* spp. Isolates within this group had a Dice similarity coefficient ranging between 0.69 and 0.96, and hence they were considered as genetically more similar with one another than those within *F. andiyazi*, and likely represent a single species. One isolate within this group had 69% similarity while the rest had at least 70% similarity between each other. The six primer combinations used in this study generated a total of 200 clearly scorable bands. Of these, 71 (35.5%) were unique to the new *Fusarium* species while 70 bands (35%) were unique to *F. andiyazi* isolates. The remaining 59 bands (29.5%) were shared across the species. Of the 71 bands unique to *Fusarium* spp., 31 (56%) were polymorphic while 60 (86%) bands unique to *F. andiyazi* were also polymorphic.

Principal coordinates analysis (PCO) also revealed the population subdivision within and between the two *Fusarium* species. Accordingly, the isolates were categorized into three groups (Figure 2). The first three principal coordinates accounted for 89.4, 4.5 and 2.3% of the total variation, respectively. PCO grouped 16 of the *F. andiyazi* isolates that formed the first two clusters of the UPGMA tree into two groups. The first group was made of 9 isolates while the second PCO group consisted of 7

### Table 1. Geographic origin of *Fusarium* isolates.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Origin</th>
<th>Altitude (m)</th>
<th>Latitude (°)</th>
<th>Longitude (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1-16</td>
<td>Welayita</td>
<td>1947-1952</td>
<td>6°58’-6°60’</td>
<td>37°51’-37°53’</td>
</tr>
<tr>
<td>Fsp1-22</td>
<td>Gidole</td>
<td>1297-1590</td>
<td>5°40’-5°42’</td>
<td>37°22’-37°24’</td>
</tr>
</tbody>
</table>
Table 2. Nucleotide sequences of adapters and primers used in the AFLP analysis.

<table>
<thead>
<tr>
<th>Adapters</th>
<th>Primer sequences</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>EcoRI</td>
<td>5’CTCGTAGACTGCGTACC3’</td>
<td>Adapter</td>
</tr>
<tr>
<td></td>
<td>CATCTGACGCATGGTTA5’</td>
<td></td>
</tr>
<tr>
<td>Msel</td>
<td>5’GACGATGAGTCTCGAG3’</td>
<td>Adapter</td>
</tr>
<tr>
<td></td>
<td>TACTCAGGACTGAT5’</td>
<td></td>
</tr>
<tr>
<td>EcoRI</td>
<td>5’GACTGCGTACCAATTC3’</td>
<td>Nonselective primer</td>
</tr>
<tr>
<td>Msel</td>
<td>5’GATGAGTCTCGAGTAA3’</td>
<td>Nonselective primer</td>
</tr>
</tbody>
</table>

Primer sequences

<table>
<thead>
<tr>
<th>Selective Primer combination</th>
<th>EcoRI (5’→3’)</th>
<th>Msel (5’→3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E19 × M15</td>
<td>GAC-TGC-GTA-CCA-ATT-CGA</td>
<td>GAT-GAG-TCC-TGA-ACA</td>
</tr>
<tr>
<td>E19 × M16</td>
<td>GAC-TGC-GTA-CCA-ATT-CGA</td>
<td>GAT-GAG-TCC-TGA-ACC</td>
</tr>
<tr>
<td>E20 × M17</td>
<td>GAC-TGC-GTA-CCA-ATT-CGC</td>
<td>GAT-GAG-TCC-TGA-ACG</td>
</tr>
<tr>
<td>E21 × M16</td>
<td>GAC-TGC-GTA-CCA-ATT-CGG</td>
<td>GAT-GAG-TCC-TGA-ACC</td>
</tr>
<tr>
<td>E21 × M17</td>
<td>GAC-TGC-GTA-CCA-ATT-CGG</td>
<td>GAT-GAG-TCC-TGA-ACG</td>
</tr>
</tbody>
</table>

Figure 1. Dendogram showing the genetic diversity of 38 isolates of two *Fusarium* species based on Dice similarity matrix of AFLP bands.
Table 3. Dice similarity index within and between *Fusarium* species.

<table>
<thead>
<tr>
<th>Species</th>
<th><em>F. andyazi</em></th>
<th><em>Fusarium</em> spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fusarium andyazi</em></td>
<td>0.391 - 0.907</td>
<td>0.044 - 0.227</td>
</tr>
<tr>
<td><em>Fusarium</em> spp.</td>
<td>0.044 - 0.227</td>
<td>0.693 - 0.957</td>
</tr>
</tbody>
</table>

Figure 2. Principal coordinates analysis of 38 *Fusarium* isolates based on AFLP fingerprints.

isolates. The 22 remaining isolates belonging to the new *Fusarium* species were aggregated within a single PCO group with only 1 isolate barely separated from the rest. These results are in line with the cluster analysis of UPGMA.

**DISCUSSION**

The *Fusarium* isolates included in the current study fulfilled the morphological characteristics of *Fusarium* as described in Leslie and Summerell (2006). However, sequence analysis revealed the presence of two *Fusarium* species associated with stalk rot in Southern Ethiopia. The current results are in line with previous works that reported the co-occurrence of different *Fusarium* spp. on the same plant (Summerell et al., 2011; Ramdial et al., 2017; Minnaar-Ontong et al., 2017). Results from sequencing confirmed the first species as *F. andyazi*, a species which was first described by Marasas et al. (2001). This species was subsequently reported to be present in different parts of the world including Australia, Ethiopia, Nigeria, South Africa and United States (Marasas et al., 2001; Marley et al., 2004; Leslie et al., 2005; Leslie and Summerell, 2006 and Summerell et al., 2011). Nevertheless, except for initial reports, no further work has been done on this particular species in Ethiopia to the best of the author’s knowledge. As a result, the diversity of this pathogen remains largely unknown to date.

Isolates belonging to the two *Fusarium* species varied not only in terms of their morphology and sequence but they also differed in growth rate, when incubated at 25 and 30°C. Isolates of *F. andyazi* grew slower than those of the newly recovered *Fusarium* species at both temperatures. Besides, the growth rate of *F. andyazi* isolates was also consistent across temperatures. Isolates of the same species showed similar growth rate at both of these temperatures in a previous study (Leslie et al., 2005). Isolates of *Fusarium* species on the other hand did not grow consistently across temperatures.

Although cultural/morphological characterizations provide a basis for both inter- and intra-species diversity studies; as suggested in other pathosystems, they may be unstable, highly influenced by the growth environments and rather change with the age of the colonies (Browning et al., 1999; Crouch et al., 2006 and Rivera-Vargas et al., 2006). As a result, such taxonomic features need to be supplemented with other characters like molecular markers that differentiate biological entities at the genetic level. Currently there is a growing
interest to assess the genetic diversity of fungi including Fusarium based on sequence analysis (McDonald et al., 2012; Leavitt et al., 2013; Maphosa et al., 2016; Larata et al., 2017).

In accordance with sequence analysis and morphocultural characterization, AFLP analysis also showed the presence of at least two genetically distinct Fusarium populations associated with sorghum stalk rot in Southern Ethiopia. The two Fusarium species were not only genetically but also geographically separated as there is more than 50 km distance between the districts from where they were obtained. Backhouse et al. (2001) and Saremi et al., (1999) have reported climatic preferences among Fusarium species from both natural and agricultural ecosystems. This report was also supported by Vigier et al., (1997); De Wolf et al., (2003) and Moschini et al. (2004) that ascertained the influence of climate and local weather variations on the recovery of Fusarium species. Both UPGMA and PCO analyses of AFLP bands suggested greater variation within F. andiyazi than within Fusarium species. Leslie et al. (2005) proposed a 40% similarity cut-off to identify strains into a single species. As the most distantly related isolates within this species had a 39% Dice similarity, which is just close to the 40% boundary, it is better not to reach a conclusion that F. andiyazi isolates belong to different species. In the current study, all isolates belonging to F. andiyazi were isolated from sorghum stalks collected from the district of Welayita, with elevation ranging from 1947 to 1952 m above sea level (masl), while those belonging to the new Fusarium species were isolated from the Gidole district, with elevation of 1297-1590 masl. Based on eleven years weather data from the National Meteorological Agency, Welayita district has a total annual rainfall of 1262 mm, and temperature of 13.6 - 23.4°C (18.5°C average). There is no reliable weather data for Gidole district. However, this relatively low lying district is known to have a more warm and humid weather than Welayita. Preparations are now underway to work on the speciation of isolates belonging to the new species, and to further characterize them on the basis of mycotoxin profiling, mating types and other characteristic features. F. andiyazi is not a known mycotoxin producer (Leslie et al., 2005). However, the toxin production potential of isolates belonging to the new Fusarium species need to be ascertained especially in light of their isolation from cereal grains. This is of paramount importance as several Fusarium spp. are known producers of mycotoxins that pose health risks to consumers of contaminated plant products (Antonissen et al., 2014; Wu et al., 2014; Van der Lee et al., 2015; Duan et al., 2016).

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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REFERENCES


Full Length Research Paper

Copper (II) ions adsorption by untreated and chemically modified *Tectona grandis* (Teak bark): Kinetics, equilibrium and thermodynamic studies

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In this study, untreated *Tectona grandis* (UTG) and citric acid-modified *T. grandis* (CAMTG) bark powder were used for the adsorption of Cu (II) ions from aqueous solution. The UTG and CAMTG were characterized by Fourier Transform Infrared (FTIR), and scanning electron microscopy (SEM). The adsorption characteristics were carried out by determining the solution pH, initial concentration of Cu (II) ions, effect of time and temperature. Langmuir, Freundlich and Temkin isotherms were used to describe the equilibrium model with Freundlich isotherm giving the best fit. The maximum monolayer adsorption capacity for CAMTG was higher than that of UTG. Also, there was about a four-fold increase in the adsorption of Cu(II) ions by CAMTG \((A_0 = 87.0 \text{ mg/g})\) over UTG \((A_0 = 22.9 \text{ mg/g})\). The kinetic data were explained by employing the pseudo-first and pseudo-second order models. The pseudo-second order kinetic model has an outstanding suitability to the experimental data. The positive enthalpy and negative free energy are indications of the endothermic and spontaneous nature of the copper (II) ion adsorption process. CAMTG is therefore, a more viable adsorbent for the removal of Cu(II) ions from aqueous solution than UTG.

**Key words:** Adsorption, copper, equilibrium, kinetics, *Tectona grandis*.

INTRODUCTION

The increased rate at which heavy metals such as copper are released into the environment in the 21st century has raised serious health concerns all over the world. The rapid and dangerous increase in the level of these heavy metals in the environment is due to the nonchalant attitudes to environmental safety by some industries involved in their production. Culpable industries in this respect are those of metallurgical, galvanizing, metal finishing, electroplating, mining, power regeneration, electronic devices manufacturing and tannery (Ajaelu et al., 2017). Copper toxicity, for instance, has been implicated in health related issues, among which are hyperactivity in children, depression, migraine, extreme tiredness, anorexia, premenstrual syndrome, depression, anxiety and learning disorder. Some of the methods for separation and recovery of heavy metals are ion exchange, chemical precipitation, electrocoagulation (Akyol, 2012), evaporation and membrane processes (Wang and Chen, 2009) which are used on a large scale. However, these procedures are inadequate and
uneconomical when metal ions exist in relatively low concentrations (Bhatti et al., 2007). They also generate large quantities of toxic sludge and secondary pollutants thereby requiring the use of large amount of reagents (Yadava et al., 2010). The removal of toxic heavy metals from industrial wastewaters using conventional chemical approaches like adsorption, oxidation and reduction and chemical precipitation, among others (Yadav, 2010), proved to be not cost effective. Similarly, activated carbon which has been employed to reduce the amount of heavy metal to harmless level due to its operational simplicity and reuse potential (Anupam et al., 2011) remains an expensive material (Mohanty, 2005). However, in recent years, the use of plant materials for the removal of heavy metal has become a more acceptable method because it has the ability to cause a reduction in the quantities of heavy metal even at low concentrations. Biosorbents that have been adopted for reducing to harmless level the heavy metals in the environment include Senna alata (Ajaelu et al., 2017), sawdust (Vaishya and Prasad, 1991), grape stalks (Villaescusa et al., 2004), carrot residue (Nasernejad et al., 2005), Ethiopian pepper (Ajaelu et al., 2011), groundnut shells (Shukla and Pai, 2005), wild herbs (Al-Sennanai and Al-Fawzan, 2018), rice shell (Aydin et al., 2007) and wine making waste (Alguacil et al., 2018). Also, our preliminary investigations revealed that T. grandis was effectual in the reduction of cadmium ions level from waste water (Ajaelu et al. 2013). Tectona grandis (teak) is a member of the Lamiaceae family. It is a large deciduous tree that is dominant in mixed hard wood forest reaching over 30 m in height in favorable conditions (Orwa et al., 2009). The plant is readily available locally.

This study, therefore, investigated the effectiveness of T. grandis (UTG) as an adsorbent for reducing the amount of Cu\(^{2+}\) ions in solutions. Two forms of the plant material —untreated T. grandis (UTG) and citric acid modified T. grandis (CAMTG) were tested. Characterization of UTG and CAMTG was done with SEM and FTIR. Equilibrium studies were explained by Langmuir, Freundlich and Temkin models. The kinetic studies were elucidated using Pseudo-first order and pseudo-second order models. Thermodynamic parameters such as free energy, entropy and enthalpy were also determined for the adsorptive reduction in the level of Cu\(^{2+}\) ions by UTG and CAMTG.

### Materials and Methods

All reagents used are of analytical grade. Citric acid monohydrate (CA) (Figure 1) was used in the chemical modification of the biomass. Stock solution of 1000 mg.L\(^{-1}\) of Cu\(^{2+}\) from Cu(NO\(_3\))\(_2\) salt was prepared. Solutions with concentrations ranging from 20 to 100 mg.L\(^{-1}\) of Cu\(^{2+}\) ions were prepared by appropriate dilution of the stock solution immediately prior to their use. The T. grandis biomass was obtained from a wetland situated at Iwo, Nigeria (7° 38' 01" N, 4° 11" 01' E). After harvest, the biomass was washed several times with deionized water to remove the dust particles, and then dried in an oven at 373K for 24 h. The dried biomass was crushed by a high speed electric grinder. The particles were sieved with a 500-μm mesh size and stored in a plastic bag.

### Citric acid modification

The chemical modification of T. grandis was similar to that already described by Vaughan et al., (2001) with little modifications. 0.2 M CA was added to UTG in the ratio of 12:1 (CA: UTG, w/v) and stirred for 45 min. The oven at 50°C was used to dry the UTG/acid mixture for 2 h. This was followed by increasing the temperature of the oven to 120°C to ensure thermochemical reaction of the mixture. The dry mixture was then cooled at room temperature, after which 0.1 M NaOH was added and agitated for 1 h to neutralize any residual acid present. The CA modified T. grandis (CAMTG) was then washed severely with de-ionized water to remove the residual alkali. The wet CAMTG was dried in an oven at 105°C until constant weight and stored in a stopped plastic tube.

### Instrumental characterization of UTG and CAMTG

Fourier Transform Infrared Spectrophotometer, Agilent Technologies Cary 630FTIR spectrometer, was used for functional group determinations on the surface of UTG and CAMTG. A sample press, which is a portion of the ATR interface, was employed to make certain that the UTG and CAMTG were in good contact with the surface of the sensor. A region of 4,000–650 cm\(^{-1}\) at 4 cm\(^{-1}\) resolution were employed to collect the data. The surface morphologies of UTG and CAMTG were determined with scanning electron microscope (Zeiss Auriga HRSEM).

### Adsorption of Safranin O

Equilibrium adsorption was determined as previously described (Ajaelu et al., 2017). In brief, batch adsorption experiments were carried out by contacting 0.5 g of CAMTG (and UTG) with 20 mL of copper solution pH in a 250-mL beaker. The samples in the beakers then agitated on an electric shaker at 298 K with a speed of 250 rpm until equilibrium was attained. Thereafter, the mixture was filtered and the concentrations of the residual Safranin O were determined using atomic absorption spectrophotometer (PG 990, PG Instruments, Britain). The amount of Cu\(^{2+}\) adsorbed, \(q_e\) (mg/g) (Equation 1) and the corresponding removal percentage (%) (Equation 2) can be calculated by the following equations:

\[
q_e = \frac{(C_o - C_e)V}{w} \tag{1}
\]

\[
\% \text{ sorption capacity} = \frac{(C_o - C_e)}{C_o} \times 100 \tag{2}
\]

Where \(C_o\) (mg/L) and \(C_e\) (mg/L) are the initial concentration of Cu\(^{2+}\) and the equilibrium concentration of Cu\(^{2+}\) in solution respectively; also, \(V\) (L) and \(w\) (g) are the volume of the Cu\(^{2+}\) and weight of either UTG or CAMTG respectively.

The effect of pH was determined from pH 2 to 8 by agitating 0.2 g of CAMTG/UTG with 20 mL of 20 mg/L solution of Safranin O dye at 298K. The reduction in the concentration of Safranin O was evaluated.

Adsorption kinetic experiments were carried out by shaking 0.3 g each of adsorbent with 120 mL of 20 - 80 mg/L Cu\(^{2+}\) solutions at pH 7 and the residual concentration was obtained. The amount of Cu\(^{2+}\)
adsorbed $q_t$ (mg/g) was obtained by the following equation:

$$q_t = \frac{C_o - C_t}{m} V$$

(3)

Where $C_t$ (mg/L) depicts the amount of Cu (II) ions adsorbed at time $t$.

Adsorption thermodynamic experiments were carried out by agitating 20 mL of the Cu$^{2+}$ solutions of varying concentrations (20-100 mg/L) with 0.1 g of CAMTG/UTG at varying temperatures (303, 308, 313 and 318K).

Theory

Adsorption Isotherm

Langmuir, Freundlich and Temkin isotherm models were employed to illustrate the observed experimental adsorption equilibrium data. The models are stated below:

(i) The Langmuir isotherm model: For monolayer adsorption, this has gained wide application to heavy metal sorption process. The linear equation for Langmuir isotherm is

$$C_e = \frac{1}{A_o b} + \frac{C_e}{A_o}$$

(4)

Where $A_o$ is the Langmuir maximum uptake capacity (mg/g) and $b$ is the Langmuir constant associated with the affinity of the binding site and the energy of adsorption in Lmol$^{-1}$, $q_e$ is the uptake capacity at equilibrium (mg/g) and $C_o$ is the equilibrium concentration of Cu$^{2+}$ ions (mg/L) in solution.

A dimensionless equilibrium parameter, $E_L$, is a necessary characteristic of Langmuir equation and is expressed as

$$E_L = \frac{1}{1 + aC_o}$$

(5)

Where $a$ is the Langmuir equilibrium constant in Lmol$^{-1}$ and $C_o$ is the initial metal concentration in (mg/L).

(ii) The Freundlich isotherm model: The Freundlich equation is an empirical equation applied to explain the heterogeneous systems and is depicted as

$$\log q_e = \log g_F + \frac{1}{p} \log C_e$$

(6)

Where $g_F$ (Lg$^{-1}$) is associated with the adsorption capacity of the adsorbent while $p$ is a Freundlich dimensionless isotherm constant related to the heterogeneity of the surface of the adsorbent.

(iii) The Temkin Isotherm Model: Temkin isotherm (Temkin, 1941) has the assumption that the adsorption heat of the molecules will experience a linear decrease rather than a logarithmic decrease with coverage. Temkin equation is also associated with the uniform distribution of binding energy (Foo and Hameed, 2010). The linear form of the equation is given by

$$q_e = \frac{RT}{B} \ln A + \frac{RT}{B} \ln C_e$$

(7)

Where $A$ is Temkin model binding equilibrium constant Lg$^{-1}$, and $B$ is Temkin equilibrium constant which corresponds to the differences in adsorption energy (kJmol$^{-1}$).

The Temkin model works on the assumption that the adsorption heat of the molecules in the layer linearly decreases with coverage owing to the interaction of the adsorbent with the adsorbate, and that the uniform distribution of the binding energies describes the adsorption.

Kinetics of adsorption

UTG and CAMTG adsorption of Cu$^{2+}$ were explained by pseudo first - order and pseudo-second order kinetic models. The linearized kinetic equation is depicted by

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t$$

(8)

where, $k_1$(min$^{-1}$) is the pseudo-first order rate constant for Cu$^{2+}$ adsorption on CAMTG and UTG.

The pseudo-second order kinetic equation is
\[
\frac{t}{q_i} = \frac{1}{k_2 q_i} + \frac{1}{q_i} \tag{9}
\]

Where \( k_2 \) (g mg \(^{-1}\) min \(^{-1}\)) is the pseudo-second order rate constant for Cu\(^{2+}\) adsorption on both CAMTG and UTG. \( \frac{t}{q_i} \) (min mg g \(^{-1}\)) is plotted against \( t \) (min) where the slope is \( \frac{1}{k_2 q_i} \) (g mg \(^{-1}\)) and the intercept is \( \frac{1}{q_i} \) (g mg \(^{-1}\)).

The kinetic models were considered acceptable through the sum of error squares (SSE) (Ng et al., 2012), the hybrid fractional error function (HYBRID) (Kumar et al., 2008) and the Marquardt’s percent standard deviation (MPSD) error function (Marquartt, 1963; Ajaelu et al., 2017). The error functions are

\[
SSE = \sum_{i=0}^{Z} \left( q_{e,exp} - q_{e,cal} \right)^2 \tag{10}
\]

\[
MPSD = 100 \left( 1 - \frac{1}{Z} \sum_{i=1}^{Z} \left( \frac{q_{e,exp} - q_{e,cal}}{q_{e,exp}} \right)^2 \right) \tag{11}
\]

\[
HYBRID = \frac{100}{Z} \sum_{i=1}^{Z} \left[ \left( \frac{q_{e,exp} - q_{e,cal}}{q_{e,exp}} \right)^2 \right] \tag{12}
\]

The kinetic fit is better when the error is low.

**RESULTS AND DISCUSSION**

**Characterization of UTG and CAMTG**

The textures of the external surfaces and morphology of UTG and CAMTG were observed by SEM as reflected in Figures 2a and 2b respectively. The UTG surface was irregular in shape and has some pores. After modification, a noticeable change was observed in the structure of CAMTG. It has broken surfaces with pores. UTG and CAMTG FTIR spectra are reflected in Figures 3a and b. The absorption at 3278 cm\(^{-1}\) for UTG corresponds to OH which was shifted to 3338.7 cm\(^{-1}\) in CAMTG after the addition of Cu\(^{2+}\) ion (Patel et al., 2007). The bands at 2920.4 cm\(^{-1}\) for UTG and 2818.6 cm\(^{-1}\) for CAMTG were associated with the presence of asymmetric -CH\(_2\) and symmetric vibration of CH\(_2\) group respectively. The bands at 1720.2 cm\(^{-1}\) for UTG and 1733.2 cm\(^{-1}\) for CAMTG represents C=O vibration of carboxylic acid. UTG shows an absorption band at 1620.1 cm\(^{-1}\) identified as N-H bend of amine (-NH\(_2\)). This was shifted to 1541.3 cm\(^{-1}\) in CAMTG after the sorption of copper (II) ion. The OH-bend of carboxylic acid on CAMTG was identified at 1438.8 cm\(^{-1}\). The –CH\(_3\) bend of alkane of CAMTG was located at 1369.8 cm\(^{-1}\). The C-O stretching vibration of COOH of UTG was identified at 1309.8 cm\(^{-1}\) which was shifted to 1317.6 cm\(^{-1}\) after the sorption of copper (II) ion in CAMTG. The peak at 1238 cm\(^{-1}\) for UTG is characteristic of a C-O stretch of carboxylic acid and was shifted to 1241.2 cm\(^{-1}\) in CAMTG. The peaks at 1181.8 and 1026 cm\(^{-1}\) for UTG were assigned to the C-F stretch of alkyl halide; but were shifted to 1157.3 and 1030.6 cm\(^{-1}\) respectively, in CAMTG.

The specific surface area \( Q \) of UTG and CAMTG were calculated from the value of \( A_o \) with \( Q \) obtained as follows:

\[
Q = \frac{N_A X A_o}{M} \tag{13}
\]

Where \( N_A \) is the Avogadro’s number, \( X \) and \( M \) are the cross-sectional area in m\(^2\)/g and the molar mass in g of the adsorbate respectively (Ali et al., 2013). The calculation of specific surface area is based on the \( A_o \) value, the atomic mass of copper, 63.5 g, and its cross-sectional area of 1.58 Å\(^2\) (the radius of Cu\(^{2+}\) ions for close packed monolayer is 0.71 Å). The specific surface area of UTG for Cu\(^{2+}\) removal was 5.42 m\(^2\)/g while that of CAMTG was 20.6 m\(^2\)/g. Thus, CAMTG has wider surface area as compared to UTG which was responsible for its effectiveness in removing more Cu\(^{2+}\) ions from solution.

**Effect of pH**

The solution pH has impactful effect on the adsorption of Cu\(^{2+}\) on CAMTG than on UTG. From the experimental results reflected in the graph in Figure 4, it was observed that for CAMTG, there was a significant increase in adsorption from pH 2 to 7, a sharp increase from pH 6 - 7 and then a decrease. Adsorption of Cu\(^{2+}\) ions by CAMTG was better at slightly acidic to neutral pH condition than for basic environment. This is because as the pH increases from acidic to neutral pH the number of negatively charged sites increases, and adsorption of Cu on CAMTG consequently increased. These may also be due to the chemical reaction and strong electrostatic interaction of the surface of CAMTG as well as Cu\(^{2+}\) ions in solution.

UTG increased slightly with pH due to weak surface - Cu (II) ions electrostatic interaction. Thus, pH had more profound effect on CAMTG than on UTG. Similar results were obtained by some researchers (Hameed and El-Khiaiy, 2008; Adebowale et al., 2014). Moreover, at lower acidic pHs the charges on the surfaces of UTG and CAMTG are positive due to the next protonation reactions of the hydroxyllic sites (1):

\[
\text{ZOH} + H^+ \rightarrow \text{ZOH}_2^+ \tag{1}
\]

As the pH increases, the adsorbent acidic sites were
Figure 2. SEM micrograph of (a) UTG (b) CAMTG.

Figure 3. The spectra of FTIR for (a) UTG and (b) CAMTG.
deprotonated owing to the surface that were negatively charged:

\[ \approx \text{ZOH} \rightarrow \text{ZO}^- + \text{H}^+ \]

The surfaces of UTG and CAMTG are represented by \( \approx \text{Z} \). Vasconcelos et al., (2008) and Tong et al. (2011) got identical results.

**Effect of initial metal concentration**

The initial concentration of the metal affects the uptake capacity of CAMTG and UTG to adsorb Cu\(^{2+}\) ions as shown in Figure 5. The sorption capacity of CAMTG for Cu\(^{2+}\) ions rose sharply with initial Cu\(^{2+}\) concentration from 20 to 40 mg/L and then increased gradually from 40 to 80 mg/L. UTG sorption capacity increased slightly with initial Cu\(^{2+}\) concentrations from 20 to 100 mg/L.

**Adsorption isotherm**

The parameters of the adsorption isotherms are reflected in Table 1. Experimental equilibrium results show that Freundlich isotherms (Figure 7) for both UTG and CAMTG (UTG, \( R^2 = 0.97 \) and CAMTG, \( R^2 = 0.99 \)) fitted best when compared to that of Langmuir in Figure 6 (UTG, \( R^2 = 0.91 \) and CAMTG, \( R^2 = 0.91 \)) and Temkin in Figure 8 (UTG, \( R^2 = 0.91 \) and CAMTG, \( R^2 = 0.88 \)).

Freundlich isotherm fitted better for CAMTG (\( R^2 = 0.99 \)) than for UTG (\( R^2 = 0.97 \)). In addition, the values of \( n \) are greater than unity for both UTG and CAMTG which indicate that the values of \( n \) are greater than unity for
Table 1. Equilibrium results for Cu\(^{2+}\) ions adsorption on UTG and CAMTG at 303K.

<table>
<thead>
<tr>
<th>Isotherm models</th>
<th>Parameter</th>
<th>UTG</th>
<th>CAMTG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( g_F )</td>
<td>8.34</td>
<td>1.69</td>
</tr>
<tr>
<td>Freundlich</td>
<td>( p )</td>
<td>2.22</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>( R^2 )</td>
<td>0.97</td>
<td>0.99</td>
</tr>
<tr>
<td>Langmuir</td>
<td>( A_0 )</td>
<td>22.9 mg/g</td>
<td>87.0 mg/g</td>
</tr>
<tr>
<td></td>
<td>( b )</td>
<td>0.53 L/mg</td>
<td>0.01 L/mg</td>
</tr>
<tr>
<td></td>
<td>( R^2 )</td>
<td>0.91</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>( RL )</td>
<td>0.018</td>
<td>0.42</td>
</tr>
<tr>
<td>Temkin</td>
<td>( A )</td>
<td>1.68</td>
<td>2.70</td>
</tr>
<tr>
<td></td>
<td>( B )</td>
<td>23.1</td>
<td>297.4</td>
</tr>
<tr>
<td></td>
<td>( R^2 )</td>
<td>0.9</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Figure 6. The plot of \( Ce/q_e \) vs \( Ce \) showing the Langmuir isotherm for the removal of Cu\(^{2+}\) by (a) UTG and (b) CAMTG.

Figure 7. Freundlich isotherm for the removal of Cu\(^{2+}\) ions by UTG and CAMTG.
both UTG and CAMTG which implied the favorability and intensity of adsorption of Cu$^{2+}$ ions on the surfaces of both UTG and CAMTG. The Langmuir maximum adsorption capacity of CAMTG (87.0 mg/g) is higher than that of UTG (22.9 mg/g). This is an increase of about four-fold in Cu$^{2+}$ ions adsorption by CAMTG over UTG, implying that the citric acid modification of the untreated T. grandis increased the number of active sites available for adsorption, and consequently enhance the electrostatic interaction between Cu$^{2+}$ ions and CAMTG. Langmuir isotherm efficiency can also be buttressed by determining whether the adsorption is favourable or not, using the separation factor, $R_L$, also known as the dimensionless equilibrium parameter. Both UTG ($R_L = 0.02$) and CAMTG ($R_L = 0.42$) have $R_L<1$. Thus the sorption of Cu$^{2+}$ ions on both UTG and CAMTG is favourable.

**Effect of adsorption kinetics**

The kinetic plots of pseudo-second order reaction is presented in Figure 9. The pseudo-first order kinetic plot gave a poor fit and, therefore, cannot be used to explain the sorption of Cu$^{2+}$ ions on both UTG and CAMTG. The kinetic results are presented in Table 2. The calculated values of sorption capacity using Equation 9 which is reflected in Table 2 for a pseudo-second order kinetic gave strong agreement with the experimental values ($q_{\text{exp}}$), and excellent results for the correlation coefficients were obtained. There was increase in the values of $q_{\text{calc}}$ as the concentration rose from 20 to 80 mg/L for both
UTG and CAMTG. Thus, pseudo-second order kinetic model was preferred in describing the Cu$^{2+}$ sorption onto UTG and CAMTG.

Moreover, only the pseudo-second order model, in which the metal binding capacity is assumed proportional to the number of active sites occupying the sorbents (UTG and CAMTG), gave a good representation of the sorption rate (Ajaelu et al., 2017; Ferreira et al., 2011).

Error Equations 10, 11 and 12 were used to describe the appropriateness of the kinetic models for the sorption of Cu$^{2+}$ ions on UTG and CAMTG. The model fits well if the error value is minimized. Table 2 showed that the SSE, HYBRID and MPSD values obtained for UTG and CAMTG were lower for pseudo-second order kinetic than for pseudo-first order kinetic models. This, of a certainty, showed that pseudo second order kinetic model described better the sorption of Cu$^{2+}$ ions on UTG and CAMTG.

### Thermodynamic effect

To study the effect of temperature, experiments were carried out at different temperatures of 303, 308, 313 and 328K and different concentrations of 20, 40, 60 and 80 mg/L, respectively. It was observed that temperature has a greater effect on CAMTG than on UTG. Moreover, at a particular temperature, concentration increase enhances the quantity of Cu$^{2+}$ ions adsorbed on the surfaces of both UTG and CAMTG.

The thermodynamic parameters were obtained from the following equations:

\[
\ln K_d = -\frac{\Delta H}{RT} - \frac{\Delta S}{RT}
\]

\[
\Delta G = -RT\ln K_d
\]

Where $K_d$, is the ratio of Cu$^{2+}$ ions adsorbed at equilibrium to that left in the solution at equilibrium. $R$ is the universal gas constant in J mol$^{-1}$K$^{-1}$, $T$ is the absolute temperature in K, $\Delta G$ (kJmol$^{-1}$) is the Gibbs free energy of adsorption, $\Delta H$ is the enthalpy change (kJmol$^{-1}$) while $\Delta S$ (Jmol$^{-1}$K$^{-1}$) is the entropy change. The various values of $\Delta H$ and $\Delta S$ were obtained from the slopes and intercepts of the plot of $\ln K_d$ against $1/T$ (as presented in Figures 10a and b) at different Cu$^{2+}$ ions concentrations of 20 to 80 mg/L and the results are listed in Table 3. It is evident from Table 3 that the sorption of Cu$^{2+}$ ions on both UTG and CAMTG are endothermic and spontaneous as reflected in the positive values of $\Delta H$ and the negative values of $\Delta G$. Thus, high temperatures enhanced the dehydration procedure and therefore, the adsorption process. Similar results were obtained by Gupta and Sharma (2002), and Chen and Wang (2006). The enthalpy changes necessary to accomplish the adsorption process was lower for CAMTG (9.23-34.5 kJ mol$^{-1}$) than for UTG (3.62-34.5 kJ mol$^{-1}$). This may be due to the existence of additional available pores for sorption in CAMTG than in UTG. The values of $\Delta G$ for both UTG and CAMTG are negative, which are indications that the sorption processes were spontaneous. $\Delta S$ was also an indication of the good affinity of adsorbent for adsorbate and increased randomness during the adsorption process (Ajaelu et al., 2017). In addition, the positive value of the entropy $\Delta S$ indicated that the increasing entropy, as a result of solvent desorption, was higher than reduction of

| Table 2. Sorption kinetic parameters for the adsorption of Cu$^{2+}$ ions on UTG and CAMTG. |
|----------------|----------------|----------------|----------------|----------------|
|                | UTG             | Pseudo first order | CAMTG       | Pseudo first order |
| $C_0$(mg/L)    | 20              | 40              | 60           | 80             | 20             | 40          | 60           | 80          |
| $q_{e\exp}$    | 3.95            | 7.9             | 11.9         | 15.8           | 1.82           | 3.77         | 5.67         | 7.22        |
| $k_1$(min$^{-1}$) | 0.014          | 0.08            | 0.01         | 0.05           | 0.015          | 0.015        | 0.01         | 5x10$^{-4}$ |
| $q_{e\calc}$  | 4.85            | 5.17            | 2.93         | 4.21           | 4.85           | 4.85         | 3.8          | 3.93        |
| SSE            | 0.64            | 1.93            | 6.31         | 8.23           | 2.14           | 0.76         | 1.32         | 2.33        |
| HYBRID         | 6.82            | 31.4            | 223.9        | 284.8          | 167.3          | 10.3         | 20.6         | 30          |
| MPSD           | 13.2            | 20              | 43.5         | 42.4           | 95.8           | 16.6         | 19.1         | 26.3        |
|                | UTG             | Pseudo Second order | CAMTG     | Pseudo Second order |
| $q_{e\calc}$  | 3.95            | 7.65            | 11.8         | 15.4           | 1.03           | 3.84         | 5.79         | 7.72        |
| $k_2$(mg$^{-1}$min$^{-1}$) | 2.64          | 1.02            | 6.97         | 11.8           | 0.53           | 7.57         | 3.37         | 2.85        |
| $R^2$          | 1               | 0.99            | 0.99         | 0.95           | 1x10$^{-3}$    | 0.99         | 1            | 0.99        |
| SSE            | 4.0X10$^{-3}$   | 0.18            | 0.08         | 0.31           | 7x10$^{-3}$    | 0.05         | 0.08         | 0.36        |
| HYBRID         | 3.0X10$^{-4}$   | 0.26            | 0.32         | 0.4            | 7x10$^{-4}$    | 0.05         | 0.08         | 0.05        |
| MPSD           | 0.09            | 1.81            | 0.52         | 1.58           | 0.2            | 1.13         | 1.17         | 3.96        |
The plot of $\ln K$ against $T^{-1}/K^{-1}$ for the sorption of Cu$^{2+}$ ions onto UTG and CAMTG.

Table 3. Thermodynamic parameters for the uptake of Cu$^{2+}$ ions by UTG and CAMTG.

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>Metal</th>
<th>$\Delta H$ (kJ/mol)</th>
<th>$\Delta S$ (J/molK)</th>
<th>$\Delta G$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc.</td>
<td></td>
<td></td>
<td>303K</td>
</tr>
<tr>
<td>UTG</td>
<td>20</td>
<td>34.5</td>
<td>126.6</td>
<td>-3.63</td>
</tr>
<tr>
<td>CAMTG</td>
<td>20</td>
<td>21.8</td>
<td>78.4</td>
<td>-3.16</td>
</tr>
</tbody>
</table>

Table 4. Adsorption capacities of various adsorbents.

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>Adsorption capacity (mg/g)</th>
<th>Temp. (K)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsicum annuum</td>
<td>28.6</td>
<td>323</td>
<td>Ozcan et al., (2005)</td>
</tr>
<tr>
<td>Irish peat moss</td>
<td>17.6</td>
<td>298</td>
<td>Gupta et al., (2009)</td>
</tr>
<tr>
<td>Wheat shell</td>
<td>17.4</td>
<td>338</td>
<td>Aydin et al., (2008)</td>
</tr>
<tr>
<td>Tamarindus indica seed powder</td>
<td>83</td>
<td>303</td>
<td>Chowdhury and Saha (2008)</td>
</tr>
<tr>
<td>Spent grain</td>
<td>10.5</td>
<td>-</td>
<td>Lu and Gib. (2008)</td>
</tr>
<tr>
<td>Garlic - treated Canna indica</td>
<td>27.9</td>
<td>-</td>
<td>Mahamadi and Chapeyama (2011)</td>
</tr>
<tr>
<td>UTG</td>
<td>22.9</td>
<td>303</td>
<td>This study</td>
</tr>
<tr>
<td>CAMTG</td>
<td>87</td>
<td>303</td>
<td>This study</td>
</tr>
</tbody>
</table>

entropy caused by solute adsorption (Vaishya and Prasad, 1991).

Table 4 shows the comparison of adsorption capacities for various adsorbents at different temperatures. It is obvious that CAMTG adsorbed best among all the other adsorbents.

Conclusion

This study examined the interaction of Cu (II) ion with the surface of untreated (UTG) and citric acid modified T. grandis (CAMTG) leaves powder. The effect of pH on the adsorption of Cu (II) by CAMTG was more pronounced than that of UTG. The surface area of CAMTG was about four-fold that of UTG. Consequently, the ratio of maximum monolayer adsorption of CAMTG to UTG is 4:1. Strong electrostatic interaction between Cu (II) ions and the adsorbents enabled pseudo-second order kinetic model to appropriately describe the adsorption of Cu (II) ions on both UTG and CAMTG at different concentrations of Cu (II) ions. Thermodynamic parameters determined showed that the metal adsorption process was endothermic and spontaneous. Citric acid modified T. grandis can be deployed to effectively reduce the amount of Cu (II) ions from aqueous solution.
CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES


Full Length Research Paper

Complete genomic sequence and recombination analysis of *wheat yellow mosaic virus* isolate from Zhouzhi in China

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Wheat yellow mosaic virus (WYMV) is the causal agent of wheat yellow mosaic disease in China. WYMV was detected in wheat sample collected from Zhouzhi of Shanxi province. The nearly complete genomic sequence of Zhouzhi isolate (WYMV-ZZ) was determined; it was compared with six complete sequences of WYMV isolates (five Chinese isolates and one Japanese isolate). WYMV-ZZ and the other six different WYMV isolates shared 96.6 to 97.7% and 95.1 to 98.2% nucleotide sequence identity for RNA1 and RNA2, respectively; at the amino acid level, WYMV-ZZ had 94.1 to 98.2% identity for RNA1 and 94.1 to 96.7% identity for RNA2, respectively, with the other six isolates. Phylogenetic analysis showed that the Nla-VPg region can separate the Chinese isolates from Japanese isolate. Based on the recombinant analysis, there were three possible recombination events; one event was located in RNA1 CI region of WYMV-ZZ with a RDP P-value of 8.526×10⁻⁶. This work advanced our understanding of the WYMV molecular variation and was helpful to study the disease spread.

**Key words:** Sequence comparison, phylogenetic analysis, Nla-VPg, recombinant event.

INTRODUCTION

*Wheat yellow mosaic virus* (WYMV), is the causal agent of wheat yellow mosaic disease of wheat in China and Japan, belongs to the genus *Bymovirus* within the family Potyviridae and is a soil-borne pathogen, it is transmitted by the fungus-like organism *Polymyxa graminis* (Sawada, 1927). In China, the disease was found in Sichuan province in the 1960s (Tao et al., 1980) and spread gradually to the middle and lower valleys of the Yangtze and Huai Rivers (Li et al., 1997; Chen, 1999). Wheat yellow mosaic virus causes typical symptoms including mosaic, yellowing, dwarving, stunting or excessive tillering, and subsequently decreasing yield. Under low-temperature conditions in the field, WYMV infects wheat. When spring comes, the infected wheat shows light green, oval- or spindle-shape spots; the temperature back to 10°C, the infected leaves show yellow mosaic symptom with the disease spots expand and emerge. Finally, WYMV causes serious damage as a result of

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yield losses (Wang et al., 2015).

The genome of WYMV is composed of two (+) single-stranded RNAs, RNA1 encodes for P3, pretty interesting Potyviridae ORF (PIPO), 7K, cytoplasmic inclusion protein (CI), 14K, nuclear inclusion protein a (Nla) which contains viral genome-linked protein (VPg) and C-terminal protein, nuclear inclusion protein b (Nlb) and coat protein (CP); RNA2 encodes for a polyprotein that contains 28- and 72-kDa proteins (Chen et al., 1999; Clover and Henry, 1999; Yu et al., 1999).

Full-length of five Chinese WYMV isolates and one Japanese WYMV isolate have been detected including isolate that came from Huangchuan, Henan province (WYMV-HC) (Yu et al., 1999); from Yangzhou, Jiangsu province (WYMV-YZ) (Chen et al., 2000); from Ya’an, Sichuan province (WYMV-YA) (Chen et al., 2000); from Japan (WYMV-JPN) (Namba et al., 1998); from Zhumadian, Henan province (WYMV-ZMD) (Zhang et al., 2010); and from Xiaqiao, Jiangsu province (WYMV-XQ with GenBank accession numbers FJ361764 and FJ361767). In this study, WYMV was detected in wheat leaves collected from Zhouzhi, Shanxi province where WYMV has not been reported before. The complete sequence of Zhouzhi isolate (WYMV-ZZ) was cloned, sequenced and compared with the other six complete sequences. Phylogenetic and recombination analyses were performed among these seven isolates.

The analysis of virus sequence and identification of virus type are helpful for breeding wheat resistant varieties (Jin et al., 2016). Full-length of five Chinese WYMV isolates and one Japanese WYMV isolate have been retrieved from GenBank database (Table 2) including isolate that came from Huangchuan, Henan province (WYMV-HC) (Yu et al., 1999); from Yangzhou, Jiangsu province (WYMV-YZ) (Chen et al., 2000); from Ya’an, Sichuan province (WYMV-YA) (Chen et al., 2000); from Zhumadian, Henan province (WYMV-ZMD) (Zhang et al., 2010); from Xiaqiao, Jiangsu province (WYMV-XQ); and from Japan (WYMV-JPN) (Namba et al., 1998).

MATERIALS AND METHODS

Sample

Wheat sample was collected from Zhouzhi, Shanxi province of China in 2008.

Total RNA extraction, reverse transcription-polymerase chain reaction (RT-PCR) and sequencing

Total RNA from wheat leaves was extracted by LiCl precipitating method (Zhang et al., 2011). Primer VP-1M was used for reverse transcription (RT) reaction and primer pair VP-1P/VP-1M was used to amplify a 704 bp fragment which was the VPg region in RNA1; primer ut-1M was used for RT reaction and primer pair ut-1P/ut-1M was used to amplify a 880 bp fragment which was the 3-termino-UTR in RNA2 (Ohto and Sakai, 2005). The purified bands were cloned into pMD18-T vector and 2-3 clones were sequenced by companies (Introigen and BIMad). The sequence fragments were combined together by DNAMAN 7.0.

Western blotting

The wheat leaves were grinded by liquid nitrogen, added 2×SDS protein buffer, blended and incubated at 100°C for 5 min, then put the samples on the ice for 5 min, and centrifuged 12,000 rpm for 10 min; the supernatant was carried onto the SDS polyacrylamide gel electrophoresis. After electrophoresis, the sample was transferred to Hybond-C membrane, and the membrane was incubated with TBST buffer (20 mM Tris, 137 mM NaCl, 0.3% Tween20, pH 7.6) containing 5% skimmed milk powder for 2 h at 37°C and added antiserum of WYMV-CP which was prepared by Yan-hong Han storing in my lab to incubate for 1 h at 37°C. Blot was rinsed by TBST for 3 times and incubated for 1 h with anti-goat IgG diluted 1:10,000. After washing in TBST, blot was visualized by NBT and BCIP (Han et al., 2002).

Phylogenetic analysis

To better understand the relationship of WYMV-ZZ and other six WYMV isolates, the full-length sequence alignments and phylogenetic analysis of nucleotide and amino acid were conducted. Phylogenetic trees were constructed for by the neighbor-joining method and visualized using MEGA X (Molecular Evolutionary Genetics Analysis version X) with 1000 bootstraps replicate (https://www.megasoftware.net) (Kumar et al., 2018).

Recombination analysis

Recombination of seven WYMV isolates was constructed by RDP4.97 (Recombination Detection Program version 4.97) (Martin et al., 2015). Various recombination detection methods were used to analyze putative recombinants and recombination breakpoints, including the programs RDP, GENECONV, BOOTSCAN, MAXCHI, SISCAN and 3SEQ. The recombination events which were surveyed by at least five different methods could be received (Zhou et al., 2012).

RESULTS

Detection of WYMV-ZZ by RT-PCR and Western blotting

WYMV-ZZ was detected from wheat sample of Zhouzhi, Shanxi province using RT-PCR and Western blotting (Figure 1).

Complete genomic sequence of WYMV-ZZ and its comparison with other six isolates

The genomic RNA sequence of WYMV-ZZ was obtained from the wheat sample by amplification of four overlapping cDNA fragments for RNA1 and two overlapping cDNA fragments for RNA2 using the primer pairs WY1001F/WY11920R, WY11858F/WY13832R, WY13578F/WY15446R, WY15378F/HC511-BHR for RNA1, and WY2001F/WY22012R, WY21927F/HC511-
Table 1. Primers used for RT-PCR and determining full-length sequences.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5'-3')</th>
<th>Genomic position</th>
</tr>
</thead>
<tbody>
<tr>
<td>WY1001F</td>
<td>AAA AATAAATAACAGACACAAACCATCAAACG (+)</td>
<td>RNA1, 1-36 nt</td>
</tr>
<tr>
<td>WY11920R</td>
<td>TGATACAGCTGGATCCGTTGC (-)</td>
<td>RNA1, 1920-1898 nt</td>
</tr>
<tr>
<td>WY1185F</td>
<td>CACGCAATGGATCCAGCTTCTCATA (+)</td>
<td>RNA1, 1858-1881 nt</td>
</tr>
<tr>
<td>WY13832R</td>
<td>CTGCCCTCCTGGCGTCTGATATCTT</td>
<td>RNA1, 3832-3808 nt</td>
</tr>
<tr>
<td>WY1357F</td>
<td>TATTGAAAGATGCTCCAGCGATG (+)</td>
<td>RNA1, 3578-3600 nt</td>
</tr>
<tr>
<td>WY15446R</td>
<td>AACTTCCCTGCTCGATGATGTCG</td>
<td>RNA1, 5446-5423 nt</td>
</tr>
<tr>
<td>WY1537F</td>
<td>ACTTCCCGCCCGAACCAGTCAGCAG (+)</td>
<td>RNA1, 5378-5401 nt</td>
</tr>
<tr>
<td>WY2001F</td>
<td>AAAATAAACCACAAAAACAAAAC (+)</td>
<td>RNA2, 1-26nt</td>
</tr>
<tr>
<td>WY22012R</td>
<td>CTGAATTGCTGCTGAGATCAGC</td>
<td>RNA2, 2102-2077 nt</td>
</tr>
<tr>
<td>WY21927F</td>
<td>GAAATTCAGAGCTCAAGCAGCTCA (+)</td>
<td>RNA2, 1927-1953 nt</td>
</tr>
<tr>
<td>HC511-BHR</td>
<td>GGATATCTGAGGATCCAGCTG (+)</td>
<td>universal primer</td>
</tr>
<tr>
<td>OligdT</td>
<td>GGATATCTGAGGATCCAGCTG (+)</td>
<td>universal primer</td>
</tr>
<tr>
<td>VPg-1P</td>
<td>TGAAGATGACTCCAGCTCAGGG (-)</td>
<td>RNA1, 3578-3587 nt</td>
</tr>
<tr>
<td>VPg-1M</td>
<td>GACCTGGGATAGGAGAATTC (-)</td>
<td>RNA1, 4281-4262 nt</td>
</tr>
<tr>
<td>ut-1P</td>
<td>CTTAGAGGTGGAGCACGGA (+)</td>
<td>RNA2, 2736-2755 nt</td>
</tr>
<tr>
<td>ut-1M</td>
<td>GACGATCGACAGGTGCATTG (-)</td>
<td>RNA2, 3595-3576 nt</td>
</tr>
</tbody>
</table>

Figure 1. Result of RT-PCR (A) and western blotting (B). Lane 1: Wheat sample from Zhouzhi; Lane 2: Healthy control; Lane 3: Mock; Lane 4: Positive control; M1: λ DNA digested by Hind III and EcoRI; M2: Protein marker (SM 0671, NEB).

BHR for RNA2 (Table 1). These primers were derived from the conserved region of six known isolates. A nearly complete nucleotide sequence of WYMV was determined, apart from short regions where the primers annealed at the 5'- and 3'-terminus. The full-length sequence of WYMV was submitted to GenBank with accession number FJ261765 for RNA1 and FJ361768 for RNA2 (Table 2).

Based on the full-length nucleotide comparison, WYMV-ZZ shared 96.6 to 97.7% and 95.1 to 98.2% nucleotide sequence identity for RNA1 and RNA2, respectively, with the other six isolates (WYMV-HC, WYMV-YA, WYMV-YZ, WYMV-XQ, WYMV-ZMD, WYMV-JPN). At the amino acid level, WYMV-ZZ had 94.1 to 98.2% identity for RNA1 and 94.1 to 96.7% identity for RNA2, respectively, with other six isolates; the identity for genes of RNA1 and RNA2 was >90% between WYMV-ZZ and six other isolates (Table 3). The nucleotide sequence identities for the individual ORFs and UTR of RNA1 between WYMV-ZZ and six other isolates were 98.0 to 99.0% for P3, 96.5 to 98.5% for 7K, 95.5 to 97.7% for CI, 93.5 to 96.7% for 14K, 96.4 to 98.9% for NIa-VPg, 96.1 to 97.1% for NIa-Pro, 96.9 to 97.5% for CP, 96.6 to 97.7 for 5' UTR and 96.5 to 98.1% for 3' UTR; for the individual ORFs and UTR of RNA2 the nucleotide sequence identities between WYMV-ZZ and six other isolates were
94.9 to 98.3% for P1, 93.8 to 98.3% for P2, 91.1 to 98.2% for 5′ UTR, and 98.2 to 99.0% for 3′ UTR. At the amino acid level, the identities for the individual ORFs of RNA1 between WYMV-ZZ and six other isolates were 96.6 to 98.8% for P3, 95.5 to 98.5% for 7K, 93.9 to 97.6% for CI, 91.9 to 97.6% for 14K, 93.0 to 98.9% for Nla-Vpg, 90.0 to 97.3% for Nla-Pro, and 98.0 to 98.6% for CP. For RNA2 the identities were 94.5 to 98.8% for P1 and 94.0 to 98.0% for P2.

Phylogenetic analysis of seven different isolates

To better understand the relationship between WYMV-ZZ and six other isolates, the phylogenetic analysis of seven different isolates was constructed. Phylogenetic trees were constructed for P3, 7K, CI, 14K, Nla-Vpg, Nla-Pro, CP, and full-length of RNA1, and for P1, P2, and full-length of RNA2 by the neighbor-joining method and visualized using MEGA (version X) with 1000 bootstrap replicates. The results showed that P3, CI, 14K, Nla-Vpg, Nla-Pro, Nlb and CP of WYMV-ZZ were more closely related to WYMV-YA, 7K of WYMV-ZZ was close to WYMV-XQ; and that P1 and P2 of WYMV-ZZ were close to WYMV-ZMD (data not shown). The full-length of WYMV-ZZ was close to WYMV-YA for RNA1 and was close to WYMV-ZMD for RNA2 (Figure 2A and B). The phylogenetic trees generated based on the Nla-Vpg region of RNA2 showed that this region clustered together with the other five Chinese isolates, while WYMV-JPN formed a distinct branch (Figure 2C and D).

Recombination analysis

The seven sequences of WYMV isolates were processed and examined for recombination at the same time. The major parent, minor parent, the event and the corresponding P-value of four recombination events are as shown in Figure 3 and Table 4. The most possible one recombination event of WYMV-ZZ, which is located in 2,598 to 4,019 nt of RNA1 CI region, may recombined with unknown major parent (WYMV-JPN) and minor parent (WYMV-HC) with a RDP P-value of 8.526×10^{-06}. The second recombination event of WYMV-HC, which is located in 564 to 894 nt of RNA2 P1, may recombined with major parent (WYMV-ZZ) and minor parent (WYMV-YA) with a RDP P-value of 5.444×10^{-05}. The third recombination event of WYMV-XQ, which is located in 1698 to 3196 nt of RNA P2 and 3′ UTR regions, may recombined with major parent (WYMV-ZMD) and minor parent (WYMV-YA) with a RDP P-value of 3.147×10^{-05}.

In this study, WYMV was detected in wheat leaves which is collected from Zhouzhi, Shanxi province of China in 2008. The wheat samples were infected with WYMV confirming by both RT-PCR and western blotting. For RT-PCR, targeting the Vpg region in RNA1, primer VP-1M was used for reverse transcription reaction and primer pair VP-1P/VP-1M was used to amplify a 704 bp fragment; targeting the 3′-termino-UTR in RNA2, primer ut-1M was used for RT reaction and primer pair ut-1P/ut-1M was used to amplify the 880 bp fragment. Using antiserum of WYMV-CP, Western blotting was carried out with the 32 kD positive band (Figure 1B).

Apart from short regions where the primers annealed at the 5′- and 3′-terminus, a nearly complete nucleotide sequence of WYMV-ZZ was determined and given the GenBank accession number FJ361765 for RNA1 and FJ361768 for RNA2, respectively (Table 2).

Based on the nucleotide sequence comparison, WYMV-ZZ shared 96.6 to 97.7% and 95.1 to 98.2% nucleotide sequence identity for RNA1 and RNA2 with the other six isolates (WYMV-HC, WYMV-YZ, WYMV-YA, WYMV-ZMD, WYMV-JPN) infecting wheat in different parts of the world. At the amino acid level, WYMV-ZZ had 94.1 to 98.2% identity for RNA1 and 94.1 to 96.7% identity for RNA2 with other six isolates. The identity for genes of RNA1 and RNA2 were >90% between WYMV-ZZ and six other isolates (Table 3).

To better understand the relationship between WYMV-ZZ and other six isolates, the phylogenetic analysis were carried out using MEGA (version X) with 1000 bootstrap replicates. The results showed that P3, CI, 14K, Nla-Vpg, Nla-Pro, Nlb and CP of WYMV-ZZ were more closely related to WYMV-YA, 7K of WYMV-ZZ was close to WYMV-XQ; and that P1 and P2 of WYMV-ZZ were close

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Place</th>
<th>RNA1</th>
<th>RNA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>WYMV-HC</td>
<td>Huangchuan, Henan Province</td>
<td>AF067124</td>
<td>AF041041</td>
</tr>
<tr>
<td>WYMV-YZ</td>
<td>Yangzhou, Jiangsu Province</td>
<td>AJ131981</td>
<td>AJ131982</td>
</tr>
<tr>
<td>WYMV-YA</td>
<td>Yaan, Sichuan Province</td>
<td>AJ239039</td>
<td>AJ242490</td>
</tr>
<tr>
<td>WYMV-ZMD</td>
<td>Zhumadian, Henan Province</td>
<td>FJ361766</td>
<td>FJ361769</td>
</tr>
<tr>
<td>WYMV-XQ</td>
<td>Xiaqiao, Jiangsu Province</td>
<td>FJ361764</td>
<td>FJ361767</td>
</tr>
<tr>
<td>WYMV-ZZ</td>
<td>Zhouzhi, Shanxi Province</td>
<td>FJ361765</td>
<td>FJ361768</td>
</tr>
<tr>
<td>WYMV-JPN</td>
<td>Japan</td>
<td>D86634</td>
<td>D866350</td>
</tr>
</tbody>
</table>
to WYMV-YA for RNA1 and was close to WYMV-ZMD for RNA2 (Figure 2A and B). The phylogenetic trees generated based on the Nla-VPg region of RNA2 showed that this region clustered together with the other five Chinese isolates, while WYMV-JPN formed a distinct branch (Figure 2C and D).

Recombination of seven WYMV isolates was analyzed by RDP4.97. Six recombination detection methods were used to analyze putative recombinants and recombination breakpoints. The programs used were RDP, GENECONV, BOOTSCAN, MAXCHI, SISCAN and 3SEQ.

Detected by at least five different methods, three recombination events were received (Zhou et al., 2012). The recombination event detected in RNA1 CI region of WYMV-ZZ, which is located in 2,598-3,344 nt, may be recombined with unknown major parent (WYMV-YZ) and minor parent (WYMV-HC) with a RDP P-value of 8.526×10^{-8}. There was no recombination event RNA2 of WYMV-ZZ. Consistent nucleotide and amino acid, and close phylogenetic relationships may imply the molecular evolution of WYMV is a genetic stability progress.

VPG is a multiple function protein, which participates in the genomic replication and interacts with 3-terminal poly-A to achieve similar function with 5-terminal cap structure (Gallie et al., 1995; MURPHY et al., 1996; Ohshima et al., 2007). So far, there was little research about WYMV-VPG, the VPG of Potato virus Y exists in different forms to exercise different functions.

DISCUSSION

The genomic RNA sequence of WYMV-ZZ was determined. Sequence comparison, phylogenetic tree and recombination analysis were performed among WYMV-ZZ and other six known WYMV isolates. Consistent nucleotide and amino acid, and close phylogenetic relationships may imply the molecular evolution of WYMV is a genetic stability progress.

Table 3. Sequence identity comparison of WYMV-ZZ with other six isolates.

<table>
<thead>
<tr>
<th>WYMV-ZZ</th>
<th>WYMV-HC</th>
<th>WYMV-YZ</th>
<th>WYMV-YA</th>
<th>WYMV-XQ</th>
<th>WYMV-ZMD</th>
<th>WYMV-JPN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nt (%)</td>
<td>aa (%)</td>
<td>nt (%)</td>
<td>nt (%)</td>
<td>nt (%)</td>
<td>nt (%)</td>
</tr>
<tr>
<td>A for full length</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNA1</td>
<td>97.0</td>
<td>94.4</td>
<td>97.3</td>
<td>96.8</td>
<td>97.2</td>
<td>96.6</td>
</tr>
<tr>
<td>RNA2</td>
<td>96.9</td>
<td>96.7</td>
<td>97.3</td>
<td>96.5</td>
<td>95.1</td>
<td>95.9</td>
</tr>
</tbody>
</table>

| B for coding region |
| P3       | 98.9    | 98.5    | 99.0    | 98.5    | 98.3    | 98.0    |
| 7K       | 96.5    | 95.5    | 98.0    | 97.0    | 98.5    | 97.5    |
| CI       | 96.4    | 96.1    | 96.7    | 95.6    | 96.8    | 95.5    |
| 14K      | 93.5    | 91.9    | 95.2    | 95.2    | 96.2    | 94.6    |
| Nla-VPg  | 98.2    | 96.8    | 98.9    | 98.4    | 98.2    | 98.4    |
| Nla-Pro  | 96.8    | 90.0    | 96.7    | 95.0    | 97.1    | 96.5    |
| Nlb      | 97.7    | 98.7    | 97.7    | 98.5    | 97.2    | 97.3    |
| CP       | 97.2    | 98.0    | 97.4    | 98.3    | 97.3    | 97.5    |
| 5′UTR    | 97.0    | -       | 97.3    | -       | 97.2    | -       |
| 3′UTR    | 98.1    | -       | 97.3    | -       | 98.1    | -       |
| P1       | 94.9    | 94.5    | 97.1    | 97.2    | 95.4    | 95.5    |
| P2       | 97.0    | 97.5    | 96.8    | 96.1    | 93.8    | 95.1    |
| 5′ UTR   | 97.1    | -       | 97.1    | -       | 91.1    | 95.8    |
| 3′ UTR   | 98.7    | -       | 98.7    | -       | 99.0    | 98.2    |
during the life cycle of virus. VPg of tobacco etch potyvirus is a host genotype-specific determinant for long-distance movement (Schaad et al., 1997; Daròs et al., 1999), and may also participate in inhibiting viral gene silencing (Germundsson et al., 2006). Sequences of VPg region were used for distinguishing two Japanese pathotypes (WYMV-Y and WYMV-M) (Ohto et al., 2005). Moreover, WYMV VPg accumulated in both the nucleus and cytoplasm of infected cells but exclusively localized in the nucleus when expressed alone in plants, and VPg interacted with WYMV coat protein (CP) and proteinase 1 (P1) proteins in vitro and in planta assays, WYMV-P1 may adjust to facilitate VPg activity through regulating VPg sub-cellular distribution (Rong, 2011). The structural and subcellular distribution of VPg protein was analysed that VPg protein contained a nuclear localization signal and a nuclear export signal, that VPg protein was detected in both cytoplasm and nucleus in virus infected leaves of wheat plant cells (Bian, 2013). In addition, the WYMV-Nib8 gene was transformed into the transgenic wheat line N12-1, and this transgenic wheat can effectively control the wheat yellow mosaic virus disease (Fu et al., 2016).

Recently, the nucleotide sequences encoding CP and VPg of WYMV collected from five provinces of China was determined; the results showed the low level of genetic diversity and inferred that the WYMV in China was genetic stability or recent emergence (Sun et al., 2013). From this study, the seven WYMV isolates showed high sequence identity comparison with nucleotide and with amino acid and VPg may be the breakthrough point of WYMV ongoing molecular evolution.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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Abbreviations

WYMV, Wheat yellow mosaic virus; WYMV-HC, WYMV
Figure 3. Recombination of WYMV analyzed using RDP4.97. A-D: BOOTSCAN plot for the recombinant of WYMV-ZZ within RNA1, of WYMV-HC within RNA2, and of WYMV-YZ within RNA2. The left and the right boundaries of the pink region indicate breakpoint positions. The yellow line is the major parent: minor parent plot, the green line is the recombinant plot; the dotted line indicates the bootstrap cut off value.
isolate of Huangchuan, Henan province; WYMV-YZ, WYMV isolate of Yangzhou, Jiangsu province; WYMV-YA, WYMV isolate of Ya’an, Sichuan province; WYMV-ZMD, WYMV isolate of Zhumadian, Henan province; WYMV-XQ, WYMV isolate of Xiaqiao, Jiangsu province; WYMV-JPN, WYMV isolate of Japan; CP, coat protein; VPg, viral genome-linked protein.

REFERENCES


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