Lasiodiplodia theobromae in the Root Rot Disease Complex of Rice

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Abstract

The study prevalence and pathogenicity of Lasiodiplodia theobromae on selected NERICA rice cultivars in rice growing farms located in two agroecological zones in southwestern Nigeria. Root and soil samples were collected from 10 farms in two rice growing local government areas in the two agroecological zones. NERICA 1, 2, 4, 5, 7, 8, 10, 11, 14, WAB 56/104 and O36 were planted in sterilized soil in the screenhouse. The plants were arranged in completely randomized design and replicated six times with two treatments. Plants were inoculated with a spore suspension from pure culture of L. theobromae or uninoculated (control). Data were collected on plant height, number of leaves and number of tillers. Symptoms of disease on leaves and roots were also scored. Incidence of root rot was higher in the rainforest agroecology compared to the derived savanna zone. Of the eight pathogens from soil and six pathogens from roots, Fusarium verticilloides and Lasiodiplodia theobromae were the most prevalent. Plants inoculated with L. theobromae were stunted, had fewer leaves and reduced chlorophyll content. L. theobromae was pathogenic on all except one of the cultivars evaluated. Only one cultivar NERICA 10 showed a level of resistance against the pathogen which was accompanied by non-significant difference in the growth parameters measured. L. theobromae is a widespread pathogen of rice involved in the root rot disease complex of rice.

Keywords: Fusarium, Leaf chlorosis; NERICA; Root rot; Upland rice

Introduction

Rice is the most widely consumed staple food for a large part of the world’s human population especially Asia where more than 90% of the world’s rice is grown and consumed [1]. In Nigeria, rice is the sixth major crop in cultivated land area after sorghum, millet, cowpea, cassava and yam [2,3]. It is the only crop grown nationwide and in all agroecological zones from Sahel to the coastal swamps. Nigeria is the highest rice producer in West Africa, producing an average of 3.2 million tons of paddy rice. Rice yield are however very low compared to the regions average [4]. This low productivity is due to various constraints among which are pests and diseases. The country is also the largest consuming nation in the region, with the growing demand amounting to 4.1 million tons of rice [4]. Nigeria is the World’s second largest rice importer, spending over US$ 300 million on rice imports annually [5]. In order to reduce expenses on rice import, and provide income for local growers, successive governments of Nigeria established initiatives for increased rice production [6]. This increase in production is likely to increase pest pressure and growers need to be aware and prepared for pest appropriate management.

Several diseases have been implicated in reduced productivity of rice. Rice can be attacked by one or more pathogens at any stage, from germination to physiological maturity. Rice can seriously be affected by a large range of pests and diseases which can destroy up to 40% of the world’s rice crops [7]. The diseases range from foliar diseases such as brown leaf spot, rice blast, bacterial blight to root diseases caused by Pythium, Fusarium and nematodes [8]. Lasiodiplodia theobromae (Syn. Botryodiplodia theobromae) and F. oxysporum have also been reported to be isolated from rice [9] although losses attributed to them have not been well described. Fusarium species have been implicated in rots and vascular wilts of various plants including rice [8], while L. theobromae is associated with postharvest degeneration of various produce [10] and root rot of several crops [11-13]. L. theobromae has a wide host range. It affects both tree crops and arable crops L. theobromae is found in the tropics and sub tropics causing numerous kinds of diseases especially rot of fruits and tuber crops during storage [14]. With the very little literature on the incidence and pathogenicity of L. theobromae on rice, this study was carried out to evaluate the incidence and pathogenicity of L. theobromae on rice cultivars in selected locations in Nigeria.

Material and Methods

Field sampling

Soil and root samples were collected from ten farms each in two rice growing zones of Oyo (Ido and Iseyin) and Ogun (Ijebu-North and Ikenne) states in southwestern Nigeria. The selected local government areas in Ogun state are in the rainforest agro-ecology while those in Oyo state are in the derived savanna. The sampling was conducted on upland rice only. Soil samples per farm were made up of 20-30 randomly taken cores. Plant roots were samples systematically along a W shaped path per farm with root samples taken at every 10 min interval. Both soil and root samples were collected in polythene bags, labeled and placed in an insulated box.

Isolation of fungi from soil and root samples of rice

In the laboratory, soil samples were thoroughly mixed and three separate samples were taken out for analysis. A sample of 10 g was added to 50 ml of sterile distilled water and agitated vigorously for 30 sec. The supernatant was immediately poured into labeled test tubes from which 1 ml was taken out with a sterile pipette and spread on pre-
prepared acidified potato dextrose agar (PDA) plates. The plates were incubated for 7 days at $28 \pm 2^\circ C$ with daily observation of colonies. Single colonies were sub-cultured in pure cultures for identification. Fungi were identified using colony characteristics, microscopic morphological characteristics in comparison with descriptors [15,16].

Root samples were washed and evaluated for rot symptoms, and then sections of roots showing symptoms were cut with a sterile scalpel. The root pieces were surface sterilized, plated in acidified PDA and incubated for 7 days at $28 \pm 2^\circ C$ with daily observation. Pure cultures were made from single colonies observed then identified as previously described. Incidence of root rot was calculated using no of samples with root rot/total number of samples collected. Frequency of occurrence of each identified pathogen was computed following the method of Francis-Otokito [9].

Evaluation of NERICA rice lines for their reaction to Lasiodiplodia theobromae

Nine NERICA (1,2,4,5,7,8,10,11,14) with WAB 56/104 and OS6 were obtained from the Africa Rice Centre. The latter two cultivars served as checks for susceptibility and resistance respectively based on their reaction to rice blast. Soil was sterilized and filled into 7 kg capacity plastic pots. Four seeds of each rice cultivar were planted per pot and thinned to two plants per pot two weeks after transplanting where necessary. A week after transplanting, Lasiodiplodia theobromae suspension from pure cultures isolated from the field sampling was inoculated into soil surrounding the rice seedlings. The suspension was prepared by adding 10 ml of sterile distilled water to 7 day old pure culture plates. The agar surface was scraped with a sterile spatula and the conidia suspension filtered through three layers of sterile cotton gauze. The concentration of spores was adjusted to $2.2 \times 10^6$ after counting spores with the aid of haemocytometer. The experiment was arranged in Completely Randomized Design (CRD) and was replicated six times. Data were collected on plant height, number of leaves, chlorophyll content and number of tillers. A disease index of root rot was determined by the length and severity of the lesion [17] using the following scale; 0=no visible lesion; 1=lesion 1 mm long; 2=lesion 1 to 5 mm long, brown; 3=lesion longer than 5 mm, dark brown; 4=root totally infected and brown; 5=root destroyed and plant dying or dead. Foliar Diseases symptoms was scored using a 0-5 where 0=No damage on the leaves; 1=1-10% damage; 2=11-20% damage; 3=21-35% damage; 4=36-60% damage; 5=60% damage. Height of plant was recorded with the aid of meter rule; number of leaves was counted on each plant. Chlorophyll content was measured with a SPAD meter. Root lesion length was measured with the aid of a flexible measuring tape and used to score for root lesion.

Data were processed using Microsoft Excel. Descriptive statistics was used for data collected from the field sampling. Quantitative data from the pot experiment was analyzed using SAS statistical package for ANOVA using the procedure for generalized linear model. The significant means were separated using LSD at 5% level of significance.

Results and Discussion

There was significantly higher incidence of root rot in the locations in Ogun state which is in the rainforest agroecological zone compared to root rot incidence in Oyo state in the derived savanna (Figure 1). Fungal pathogens are favoured in warm humid conditions which is the predominant environmental condition in both locations of Ogun state [18]. This maybe the reason for the higher incidence of root rot encountered in the area. In comparison, the other locations are in the less humid plains of the derived savanna.

Pathogens identified in soil samples were Aspergillus niger, A flavus, Fusarium verticilloides, L. theobromae, Macrophoma sp. Sclerotium rolfsii, Rhizopus stolonifer and Alternaria sp (Figure 2). The same organisms were found associated with the roots except for Macrophoma sp. and Alternaria sp. A. niger was the most often encountered in the soil and the least encountered was Alternaria sp. whereas in the roots L. theobromae followed by F. verticilloides were the most frequently encountered at 62% and 58% respectively. These pathogens have been previously listed as associated with rice plants growing on the field [9,19]. The high level of occurrence of F. verticilloides and L. theobromae could be responsible for the incidence of root rot in all the locations. Both pathogen species were the second most frequently occurring pathogen in root rot disease complex of cassava [20]. Especially as these two pathogens were found in the majority of the locations sampled. In addition they were found associated with roots of the rice plants. The higher occurrence in roots rather than soil also indicates their association with roots as possible causal agents of root the rot observed.
from the significantly lower chlorophyll content measured in infected plants. _Lasiodiplodia theobromae_ has been known as an important pathogen of root and tuber crops and stored produce in the tropics [14]. The awareness of its pathogenicity on various crops has been increasing as seen in various first reports [12,13,21]. The list of host of the fungus also includes banana, mangoes, cocoa [14], cassava, yams [22], peanut [23], coffee and coconut [24].

### Table 1: Plant height, number of leaves and number of tillers of rice cultivars inoculated with _Lasiodiplodia theobromae_.

Plant height, number of leaves and number of tillers of plant inoculated with _L. theobromae_ were generally lower than the uninoculated rice cultivars (Table 2). Without inoculation with _L. theobromae_, NERICA 15, NERICA 7 and WAB 56-104 were the tallest plants while NERICA 14 was the shortest. Among the inoculated plants, however, NERICA 10 was the tallest followed by NERICA 4. Significant reduction in plant height in _L. theobromae_ inoculated plants occurred in NERICA 1, 11, 15, 7, and 8, in addition, both checks also showed stunting when inoculated. All plant except for NERICA 10 had significantly fewer leaves and number of tillers. Plant height and other growth parameters are generally impacted negatively when root function is affected. Impairment of root function caused by root rot results in the inability of the plant to carry various physiological functions leading to reduced measurement of growth parameters. Root rot of cucumber when not managed caused a significant reduction of all growth parameters [25].

| Cultivar      | Plant height (cm) | No. of leaves | No. of tillers |
|---------------|-------------------|---------------|---------------|
|               | Control           | _L. theobromae_ | Control       | _L. theobromae_          | Control       | _L. theobromae_ |
| NERICA 1      | 29.04             | 18.16         | 18.1          | 8.89*                    | 7.02          | 3.54*          |
| NERICA 10     | 28.49             | 25.94         | 17.92         | 14.88                    | 6.76          | 4.67           |
| NERICA 11     | 30.23             | 6.38         | 20.7          | 2.06*                    | 8.83          | 1.15*          |
| NERICA 14     | 23.85             | 21.86         | 16.92         | 11.56*                   | 7.06          | 3.78*          |
| NERICA 15     | 40.97             | 20.60         | 26.02         | 9.91*                    | 9.63          | 2.69*          |
| NERICA 2      | 30.84             | 17.21         | 20.69         | 10.38*                   | 8.37          | 4.59*          |
| NERICA 4      | 28.71             | 23.21         | 18.8          | 11.60*                   | 7.98          | 4.54*          |
| NERICA 7      | 40.69             | 17.91         | 25.77         | 9.79*                    | 10            | 3.29*          |
| NERICA 8      | 27.36             | 12.27         | 14.89         | 7.73*                    | 6.31          | 3.65*          |
| OS6           | 28.53             | 10.19         | 15.19         | 3.60*                    | 9.06          | 0.83*          |
| WAB 56-104    | 38.86             | 20.72*        | 23.09         | 11.26*                   | 10.37         | 4.11*          |
| LSD           | 3.75              | 3.8           | 2.48          | 2.37                     | 0.9           | 0.85           |

Values are means of six replicates per treatment with two stands per replicate. * = significant difference within the row and per parameter at 5% level of significance using LSD.

### Table 2: Effect of Lasiodiplodia theobromae on plant height, number of leaves and number of tillers of selected NERICA cultivars.

Inoculated plants showed various levels of both foliar and root symptoms of infection (Table 3). Foliar symptom of _L. theobromae_ was lowest in NERICA 10 compared to all other cultivars including the checks. The foliar symptoms began as chlorosis on the leaf tips which later turned necrotic with an active chlorotic border. NERICA 10 also showed the lowest root lesion score compared to the other cultivars and it was not significantly different from the uninoculated treatment.

Observations on the reaction of NERICA 10 in terms of the growth parameters and disease response indicated that it possesses an appreciable level of resistance to _L. theobromae_. Evidences from this study shows that _L. theobromae_ is pathogenic on several rice cultivars and is implicated in the root rot complex of rice. The pathogen is reported to be a tropical and subtropical cosmopolitan soil borne fungus [26]. It causes black rot on roots of strawberries, which led to...
wilting and eventually to blackened, necrotic discoloration in the crown of daughter plants [13], in Yucca plants it causes leaf tip dies back [21] a symptom similar to the one observed on the rice plants in this study. L. theobromae is also involved in a complex of root diseases affecting rice along with other pathogens [19,27].

| Cultivar | Foliar symptoms | Root lesion |
|----------|-----------------|-------------|
|          | Control         | L. theobromae | Control | L. theobromae |
| NERICA 1 | 0.5             | 2.8*         | 0.2     | 3.2*         |
| NERICA 10| 0.8             | 1.8*         | 0.2     | 0.7          |
| NERICA 11| 0.7             | 2.5*         | 0.5     | 0.3*         |
| NERICA 14| 0.5             | 3.0*         | 0.2     | 3.8*         |
| NERICA 15| 1.1             | 2.7*         | 0.7     | 3.0*         |
| NERICA 2 | 0.6             | 3.3*         | 0.5     | 3.5*         |
| NERICA 4 | 0.7             | 3.2*         | 0.2     | 4.2*         |
| NERICA 7 | 1               | 2.9*         | 0.3     | 3.5*         |
| NERICA 8 | 0.6             | 2.5*         | 0.7     | 3.0*         |
| OS6      | 0.5             | 2.9*         | 0.2     | 1.7*         |
| WAB56-104| 0.8             | 3.2*         | 0.3     | 3.3*         |
| LSD      | 0.13            | 0.52         | 0.12    | 0.61         |

Values are means of six replicates per treatment. * signifies a significant difference within the row and per parameter at 5% level of significance using LSD. Foliar Diseases symptoms scored from 0-5 using: 0=No damage on the leaves; 1=1-10 % damage; 2=11-20% damage; 3=21-35% damage; 4=36-60% damage; 5= >60% damage; Root lesion damage scale was 0=no visible lesion; 1=lesion 1 mm long on few roots; 2=lesion 1 to 5 mm long on few roots, brown; 3=lesion longer than 5 mm dark brown on many roots; 4=root totally infected and brown; 5=root destroyed and plant dying or dead.

Table 3: Disease severity caused by Lasiodiplodia theobromae on selected NERICA cultivars.

This study presents the presence of L. theobromae in the root rot disease complex of rice in the majority of rice growing locations sampled in south western Nigeria. It is also a confirmation of the pathogenicity of L. theobromae on rice cultivars with an attendant reduction in growth parameters of rice and possibly yield. This should be put into consideration in developing any management strategy for rice diseases.

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