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# Pathogenicity of Fungi Associated with Amaranth Cultivation in Kombé, Republic of the Congo

**Dalcantara Liana Ongouya Mouekouba[1*], Grace Jokael Etou Ossibi[1,2], Alaric Makoundou[1], Horta Rovicia Gackosso[1], Joseph Mpika[1], Attibayeba[1]**

[1]Laboratory of Biotechnology and Plant Production, Faculty of Science and Technology, Marien Ngouabi University, Brazzaville, Republic of the Congo
[2]Biology Center, Faculty of Applied Sciences (FSA), Dénis Sassou N'guesso University, Kintélé, Republic of the Congo
Email: *makanga.liana@gmail.com

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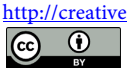




## Abstract

Amaranth is a leafy vegetable highly valued for its nutritional benefits. In the Republic of the Congo, particularly in the city of Brazzaville and the Kombé locality, market gardeners report that amaranth cultivation is threatened by fungal diseases caused by various fungi. The objective of this study was to assess the pathogenicity of fungi associated with *Amaranthus hybridus* grown in Kombé, Republic of the Congo. A survey was conducted in the Kombé area of Brazzaville to collect amaranth plants exhibiting symptoms of rot. The fungi linked to these symptoms were isolated, and a pathogenicity test was performed with the collected fungi. During the survey, five distinct types of symptoms, not previously observed at Agricongo in Kombé, appeared on healthy amaranth plants treated with conidia from various fungal genera. These symptoms included: the appearance of brown circular spots on the leaves; yellowing, deformation, and drying of the leaves; rotting and generalized wilting of the foliage; and rotting of the stem from the bottom to the top of the plant. The pathogenicity test conducted in this study on the amaranth plants yielded inconclusive results. Consequently, the pathogenicity of the identified fungi on amaranth crops could not be confirmed. This study represents the first of its kind in the area and could serve as a foundational reference for future efforts to control fungi responsible for diseases in amaranth cultivation. Given the importance of the disease, it is essential to identify and molecularly characterize these fungi.

## Keywords

Amaranth, Fungus, Symptoms, Kombé

## 1. Introduction

Amaranth is a perennial herbaceous plant of tropical origin, belonging to the *Amaranthaceae* family, and is commonly used as a leafy vegetable. It has been naturalized in central Asia and possibly Iran [1], with a cultivation history spanning over 2,000 years [2]. The cultivation of various Amaranthus species is becoming increasingly significant in Nigeria and other parts of the African continent, where the leaves of these species are grown for consumption [3]. However, amaranth production is not immune to pest attacks, which are significant factors limiting its yield [4]. Losses to parts of the plant, or even the entire plant, are caused by external agents such as biological pests. Fungi, in particular, are a major cause of disease in amaranth, responsible for approximately 70% of crop diseases [5]. The primary fungal diseases affecting amaranth are amaranth mould and damping-off. Amaranth mould, caused by the fungus *Choanephora cucurbitarum*, has been responsible for necrosis on the leaves, young stems, and petioles of amaranth in Congo since 1990 [6].

Although losses in amaranth production due to fungal diseases have been widely reported on market garden sites in the Republic of the Congo in general and in the city of Brazzaville in particular, very little work has been done on characterising the fungi responsible for these production losses.

In fact, the research conducted by [7] identified six genera of saprophytic fungi in the Kombé soil sample (*Botrytis cineara, Rhizoporus sp*, *Penicillium sp*, *Mucor sp*, *Scedospirium sp and Curvularia sp*) and also showed the presence of five isolates of unidentified endophytic fungi from the leaf tissue of amaranth grown in Kombé, using a technique based on color distribution and macroscopic appearance of the isolates. The objective of this study is to determine the pathogenicity of these fungi and to set up a database for possible control of the fungi responsible for amaranth production losses on market gardening sites in the Republic of the Congo in general and in the city of Brazzaville in particular.

## 2. Materials and Methods

### 2.1. Plant and Biological Materials

The biological material used in the study consisted of *Amaranthus hybridus* seeds. Additionally, fungi were isolated from the leaf tissue of amaranth plants cultivated in Kombé. These fungi were classified into five groups based on their macroscopic appearance and coloration, and were subsequently used for pathogenicity testing.

### 2.2. Methods

- **Sampling**

Samples were collected from 25 randomly selected growers at the cultivation site, all of whom primarily grow amaranth. They were interviewed using a semi-structured questionnaire. The questionnaire administered focused on the following parameters: characterisation of the farm and health assessment at the survey site.

Each plot was carefully examined to assess the number of plants attacked by disease and the extent of damage. Amaranth plants exhibiting symptoms of fungal attack were selected for detailed laboratory examination. A total of 15 amaranth plant samples were taken from the site and stored at $4°C$ for four weeks prior to analysis. This period covers the time needed to prepare the site and set up the nursery.

- **Isolation of Fungi Associated with Symptoms**

The fungal strains were isolated from explants taken from symptomatic amaranth plants grown in Kombé. The fungi were cultured on Malt Agar medium. To prepare the medium, 10 g of agar and 20 g of malt were mixed in a 1,000 mL Erlenmeyer flask, suspended in 500 mL of distilled water, and autoclaved at 121˚C for 15 minutes. Plant explants (leaf fragments, roots, and crowns) were inoculated into Petri dishes containing Malt Agar medium, following the method outlined by [7].

- **Identification of Associated Fungi**

The fungal strains were identified by examining their cultural characteristics, including coloration, growth pattern, and the number of mycelial colonies. A macroscopic analysis of 7-day-old fungal strains was conducted, which led to the identification of five distinct groups of fungal strains: fungus A: chA, fungus B: chB, fungus C: chC, fungus D: chD, fungus E: chE).

- **Site Preparation**

Site preparation began with the clearing and demarcation of the experimental plot. This was followed by ploughing and making the beds (**Figure 1**).

**Figure 1.** Preparation and delimitation of the experimental plot.

- **Setting Up the Nursery**

Before the trial was established, the nursery was placed in 10-liter cut-out plastic pots, positioned high on the experimental site. The soil used for the nursery was

sterilized with hot water in the plastic pots and covered with a black plastic sheet for 48 hours. The pots were then left to cool for 24 hours before sowing. Sowing was carried out in rows, with three seeds per pot. Each pot received 1 liter of water daily. Hoeing was done superficially once, on the 10th day, to promote water infiltration.

- **Trial Setup**

The experimental setup for the trial followed a completely randomized block design, consisting of three blocks and six treatments, including the control, each with three replicates. The treatments were as follows: G0 (control), G1 (chA), G2 (chB), G3 (chC), G4 (chD), and G5 (chE). Each block contained six beds, each measuring 1.2 m wide by 1.4 m long (1.68 $m^2$), separated by 0.5 m. The plants were transplanted at a spacing of 20 cm between plants in the row and 20 cm between rows, resulting in 42 plants per bed, with a planting density of 250,000 plants per hectare (**Figure 2**).

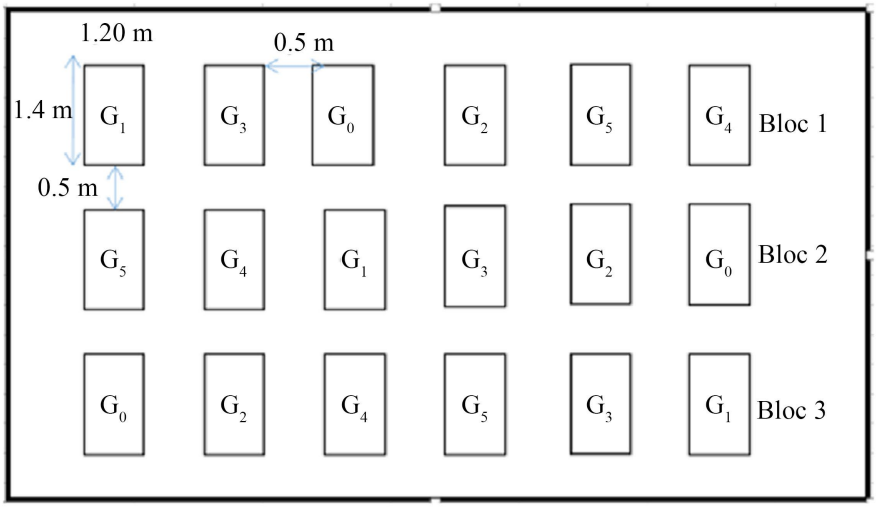



**Figure 2.** Experimental setup.

After the site preparation work, the amaranth plants were transplanted 16 days after sowing in the nursery. Transplanting occurred on June 2nd in the late afternoon to minimize sun exposure and prevent damage to the plants. The plants were not replaced after transplantation.

- **Pathogenicity of Isolated Fungi**

The fungi stored in the refrigerator were grouped into five categories based on their macroscopic appearance. One fungus from each group was selected for further cultivation. For the pathogenicity test, pure cultures of each isolated fungus, aged seven days, were suspended in approximately 4 mL of sterile distilled water. The mycelium was then gently scraped with a sterilized brush to break up the fruiting bodies. The resulting conidial suspension was collected in a sterile test tube in a fume hood, filtered, and concentrated to its maximum density.

Three (3) inoculation methods were employed: drip inoculation, local infiltration [8] and gentle inoculation.

- **Drip inoculation**: 1 mL of the conidial suspension was applied drop by drop to the base of the third leaf using a 5 $cm^3$ syringe. The leaf was superficially wounded with a sterile needle before inoculation;
- **Local infiltration**: A 5 $cm^3$ syringe was used to infiltrate 0.1 mL of the conidial suspension into the plant's neck;
- **Gentle inoculation**: 50 mL of the conidial suspension was sprayed onto the entire plant (leaf and crown).

All inoculations were performed in the field on healthy amaranth plants that were 15 days old after transplanting (JAR). Symptom development was assessed 15 days after the fungal inoculation for each treatment. The observations focused on describing the symptoms and comparing them with those observed at the Kombé center.

- **Abundance of Isolation**

The abundance of fungal genera isolation was determined by counting the Colony Forming Units (CFU), calculated using the following formula:

$$\text{CFU} = \frac{\text{Number of colonies} \times \text{Final dilution factor}}{\text{Dry weight of plant material}} \quad (1)$$

## 3. Statistical Analysis

The data collected in the field were analyzed using computer-assisted statistical tools, specifically SPSS (Statistical Package for the Social Sciences) version 22.0 and R version 4.1.3. The statistical methods applied were chosen based on the sampling methods and experimental setup used.

The normality of the residuals and the homogeneity of the variances were assessed.

The statistical techniques employed included two-factor ANOVA and linear correlations. Means were compared using the Student-Newman-Keuls test and the Kruskal-Wallis test, both at a 5% significance level.

## 4. Results and Discussions

### 4.1. Results

- **Characterisation of Isolated Fungi**

After incubation, a variety of mycoflora developed. The purity of the strains was checked through successive subculturing on different media. Subculturing involved removing a mycelial fragment with a sterile loop and transferring it to the malt agar medium. The inoculum was placed at the center of the dish. The Petri dishes were incubated in an inverted position at 28˚C for 72 hours. Based on color and macroscopic observations, the isolates were divided into five fungal groups or genera.

The strains of fungus A formed mycelial colonies with a green color. The mycelial colonies of fungus B were brown. Strains of fungus C had red-colored mycelium. The strains of fungus D produced white mycelial colonies with a filamentous appearance and a grayish underside (**Figure 3**).

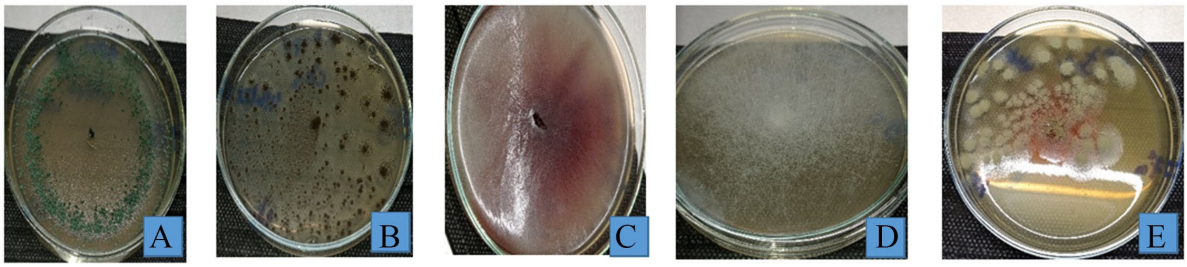
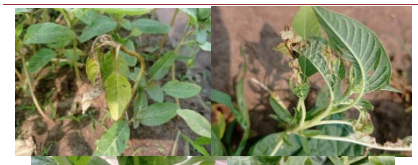



**Figure 3.** Macroscopic aspects of the five endophytic isolates used in the pathogenicity test [7].

**Table 1.** Amaranth disease: symptoms observed on *Amarantus* plants after inoculation.

| Image of the disease | Symptoms | Type of disease |
|---|---|---|
|   | Stem and leaf necrosis, chlorosis on leaves and overturning of necrotic leaves | Fungal disease caused by scale insects |
|  | Leaf turning, blistering and necrosis of leaf | Viral disease |
|  | Leaf necrosis | Fungal disease |
|  | Chlorosis at the ends of the leaves, brown spots and leaf turning | Abiotic disease: calcium deficiency |
|  | Stem rot | Fungal disease: *Choanephora cucurbitarum* |

- **Symptoms Observed**

In addition to the symptoms observed at Agricongo de Kombé, five distinct symptoms were noted on healthy amaranth plants treated with conidia of the fungal genera. These included: the appearance of brown circular spots on the leaves; yellowing, deformation, and drying of the leaves; rotting and widespread wilting of the foliage; and rotting of the stem from the bottom to the top of the plant. However, the pathogenicity test conducted in the field on healthy amaranth plants using conidial suspensions at maximum concentration did not yield conclusive results for all isolates. The five fungal isolates used in the test did not cause symptoms similar to those observed at the Agricongo center in Kombé (Table 1).

- **Abundance of Fungi Isolated from Leaflet Samples**

The results presented in Figure 4 show the isolation of five different types of fungi from the leaves of amaranth plants grown at Agricongo "Kombé". The chA fungus was the most abundant on the leaves, with an average concentration of $8333 \times 10^6$, followed by chB ($5333 \times 10^6$), chC ($4003 \times 10^6$), chD ($4000 \times 10^6$) and chE ($2000 \times 10^6$), which were the least abundant on the leaves. However, the statistical analysis shown in Table 2 reveals that there is a significant difference between the CFU concentration of the different types of fungus found on the leaves of amaranth plants grown at Agricongo in Kombé (p-value > 0.05). The analysis of variance revealed three (III) homogeneous groups (a, b, c), the most significant being those of the chA fungus (group a) and the chB fungus (group b) (Table 2).

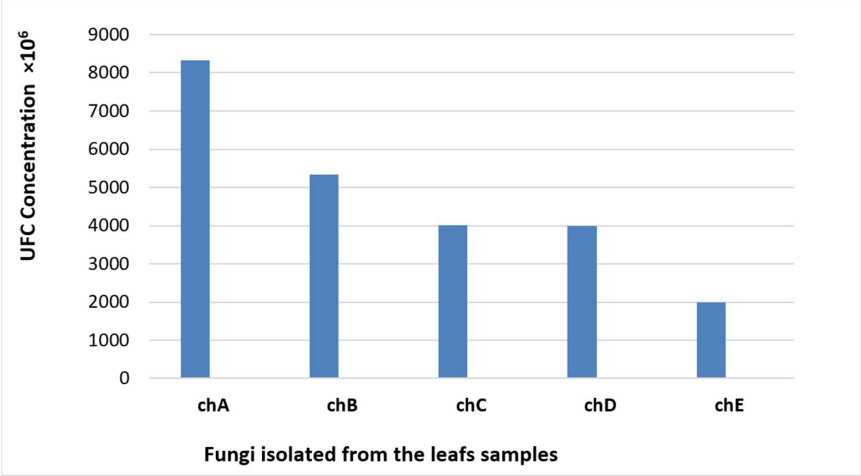



Figure 4. Abundance of fungi isolated from leaf samples.

Table 2. Abundance of fungi isolated from leaf samples.

| Fungus | UFC × $10^6$ |
|---|---|
| chA | 8333.33[a] |
| chB | 5333.33[b] |
| chC | 4003[c] |
| chD | 4000[c] |
| chE | 2000.37[d] |

CFU × $10^6$= number of fungal cells per fungus. Numbers followed by identical letters are not statistically different at the threshold of $p > 0.05$, p-value = 0.7002 according to Kruskal-Wallis; Ch: fungus (A, B, C, D, E).

- **Pathogenicity of Isolated Fungi**

The pathogenicity test conducted in the field on healthy amaranth plants using conidial suspensions at the maximum concentration did not yield conclusive results for any of the isolates. The five fungal isolates tested did not cause symptoms resembling those observed at the Agricongo center in Kombé (**Table 1**).

## 4.2. Discussions

Five different types of rot were observed on Amaranthus hybridus amaranth plants grown in Agricongo in Kombé, Republic of the Congo [7].

In our study, a pathogenicity test was conducted on healthy amaranth plants in the field using the five types of strains isolated. The results led to the appearance of symptoms that differed from those observed at Agricongo in Kombé. The five isolates used in our test, through drip inoculation, local infiltration, and spraying conidial concentrations of the fungal genera on healthy amaranth plants, caused the following symptoms: brown circular spots on the leaves, yellowing, leaf deformation, drying of the leaves, widespread wilting of the foliage, and stem rot that progressed from the bottom to the top of the plant. Overall, our test produced inconclusive results. This can be attributed to several factors, including the time delay between sample collection and inoculation in the field, as well as the presence of multiple fungi associated with the samples. In fact, our experiments were carried out in open fields close to other experimental crops.

In addition, symptoms other than those observed in Agricongo during our study could be linked to environmental factors such as: climate, rainfall and temperature, as reported by [9]. The differences in symptoms after inoculation can be explained by contamination, which could be due to the action of rain, which may have transported the fungus spores born in the soil over long distances. As stated by [10] [11], soil-born fungi disseminate the best through drainage or surface runoff during rain periods.

According to [12], one lesion can be provoked by one pathogen species that can be there after colonized by others. Similarly, our experiments were conducted in open fields near other experimental crops. The development of these other symptoms in our experiments could be caused by the presence of one or more sources of infection present on neighbouring fields. Also, attack by different pathogen agents can result in similar symptom and attack by a specific pathogen agent can result in variable symptoms, owing to the conditions [13]. Various facultative pathogens can induce similar symptoms.

The appearance of additional symptoms after inoculation of conidial suspensions during our study aligns with the findings of [14], who stated that under natural conditions, other fungi may establish themselves following the primary pathogen. These results contrast with those of [15], who conducted a pathogenicity

test on healthy tomato plants in pots using conidial suspensions ($10^5$ conidia/mL). The spray inoculation of conidial concentrations onto healthy tomato seedlings led to the development of leaf symptoms, confirming the pathogenicity of these fungi on the tomato accessions used in their test.

## 5. Conclusion

The aim of this study was to assess the pathogenicity of five fungal genera isolated from the leaves of amaranth plants grown at Agricongo "Kombé". The pathogenicity tests conducted on healthy amaranth plots during the study yielded inconclusive results, as no symptoms similar to those observed at the Kombé site were identified. Consequently, the pathogenicity of these fungi on the amaranth plots could not be confirmed, and the postulate remains unverified. Given the significance of the disease, molecular characterization of the species is essential. The aim of this study was to assess the pathogenicity of five fungal genera isolated from the leaves of amaranth plants grown at Agricongo "Kombé". The pathogenicity tests conducted on healthy amaranth plots during the study yielded inconclusive results, as no symptoms similar to those observed at the Kombé site were identified. Consequently, the pathogenicity of these fungi on the amaranth plots could not be confirmed, and the postulate remains unverified. Given the significance of the disease, molecular characterization of the species is essential so that we can set up a database to combat these fungi.

## Authors' Contributions

This work was carried out in collaboration among all authors. Author Joseph Mpika designed the research project, executed the project and edited the manuscript. Author Horta Rovicia Gackosso conducted the field activities of the project and analysed the data associated with Author Grace Jokael Etou Ossibi. Author Dalcantara Liana Ongouya Mouékouba wrote the manuscript and supervised the associated field work with Author Alaric Makoundou. Author Attibayeba is the head of the laboratory. All authors read and approved the final manuscript.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

## References

[1] Schröder, J. (2005) Revisiting the Agronomic Benefits of Manure: A Correct Assessment and Exploitation of Its Fertilizer Value Spares the Environment. *Bioresource Technology*, **96**, 253-261. https://doi.org/10.1016/j.biortech.2004.05.015

[2] Srivastava, R. (2011) Nutritional Quality of Some Cultivated and Wild Species of *Amaranthus L. International Journal of Pharmaceutical Sciences and Research*, **2**, 3152-3156. https://www.researchgate.net/publication/267960456_Nutritional_quality_of_some_cultivated_and_wild_species_of_Amaranthus_L

[3] O'Brien, G K. and Price, M.L. (2008) Amaranth: Grain and Vegetable Types. Echo

Technical Note.
https://www.el-pan-alegre.org/Amaranth_Grain-and-Vegetable_Types.pdf

[4] Jame,B., Atcha-Ahowé, C., Godonou, I. and Baimey, H. (2015) Integrated Pest Management in Vegetable Production: A Guide for Extension Agents in West Africa. International Institute of Tropical Agriculture. 120.
https://core.ac.uk/download/pdf/132673969.pdf

[5] Benhamou, N., Rey, P., Chérif, M., Hockenhull, J. and Tirilly, Y. (1997) Treatment with the Mycoparasite *Pythium oligandrum* Triggers Induction of Defense-Related Reactions in Tomato Roots When Challenged with *Fusarium oxysporum* F. Sp. *Radicis-Lycopersici*. *Phytopathology*, **87**, 108-122.
https://doi.org/10.1094/phyto.1997.87.1.108

[6] Makambila, C. and Goma, J.B. (1993) "Choanephora Cucurbitacearum" Curr. a New Fungal Pathogen of Amaranth in Congo. *Agriculture Notebooks*, **2**, 217-219.

[7] Ongouya Mouekouba, D.L., Gackosso, H.R., Makoundou, A., Mpika, J. and Attibayeba (2024) Determination of the Prevalence of Fungi Associated with Amaranth Cultivation in the Locality of Kombé, Republic of Congo. *World Journal of Advanced Research and Reviews*, **23**, 1261-1271. https://doi.org/10.30574/wjarr.2024.23.3.2763

[8] Gaitán-Chaparro, S., Navia-Rodríguez, E. and Romero, H.M. (2021) Assessment of Inoculation Methods of Thielaviopsis Paradoxa (de Seynes) Höhn into Oil Palm Seedlings under Greenhouse Conditions. *Journal of Fungi*, **7**, Article 910.
https://doi.org/10.3390/jof7110910

[9] Anahosur, K.H. (1992) Sorghum Diseases in India: Knowledge and Research Needs. Sorghum and Millets Diseases: A Second World Review. Patancheru International Crops Research Institute for the Semi-Arid Tropics, India, 45-56.

[10] Bieysse, D., Bella, M., Mouen, B., Ndeumeni, J.P., Roussel, V., Fabre, J.V. and Berry, D. (2002) Anthracnose of Berry. A Potential Threat for Global Crop of Arabica. CIRAD-CP, 144-156. https://agritrop.cirad.fr/508312/

[11] Oostland, C. and Pacico, R. (2007) For Profitable of Cereal Growing. 36-37.
http://www.agro.basf.be

[12] Mouria, A., Ouazzani, T.A.and Douira, A. (2003) Study of Certain Factors Favouring the Maintenance of the Antagonistic Activity of *Trichoderma harzianum* towards *Helminthosporium oryzae* on Rice Leaves. *Research Papers*, **5**, 50-66.

[13] Koffi, A.M.H., Atta, D.H. and Zoro Bi, I.A. (2013) Identification of Fungus Flora Associated with *Lagenaria siceraria* (Molina) Standl in Côte d'Ivoire. *Asian Journal of Agriculture and Rural Development*, **3**, 767-779.
https://ageconsearch.umn.edu/record/198253/?v=pdf

[14] Koffi, A.M.H. (2015) Pathogenicity and Genetic Diversity of Fungal Parasites of Seeds and Leaves of *Lagenaria siceraria* (Molina) Standl (Curbitaceae) from Five Localities (Alépé, Bondoukou, Bongouanou, Divo and Korhogo) in Côte d'Ivoire. Master's thesis, University Nangui Abrogoua.

[15] Djamala, S. (2017) Phytosanitary Monitoring of Tomato (*Solanum lycopersicum* L.) Accessions in the Hot and Wet Seasons at the Farako-Bâ Station. Master's Thesis, Nazi Boni University.
https://www.beep.ird.fr/greenstone/collect/upb/index/assoc/IDR-2017-SIB-SUI/IDR-2017-SIB-SUI.pdf