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# PHENOTYPIC CHARACTERIZATION OF SOME ISOLATES OF *PHAKOPSORA PACHYRHIZI* IN CAMEROON

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## ABSTRACT

The development of a rational and efficient control strategy against Asian rust caused by *Phakopsora pachyrhizi* requires knowledge of the pathogenicity and local variability of the pathogen. The general objective of this study was to phenotypically characterize some isolates of *P. pachyrhizi* in Cameroon. In five agro-ecological zones (AEZ), 34 isolates of *P. pachyrhizi* were collected from diseased leaflet samples of soybean taken in the field. The length (L) and width (w) of the phenotypic forms of different uredospores of the isolates obtained were observed and measured under the light microscope. The length to width ratio (L/w) was calculated and the isolates aggressiveness was evaluated. Microscopic observation revealed seven different uredospore shapes including two new ones (seedlike and angular). Uredospore lengths ranged from 25.40 to 27.64 µm; widths from 16.64 to 19.61 µm; and L/w ratio from 1.45 to 1.57. The pathogenicity test on detached leaflets showed that the *P. pachyrhizi* isolates obtained were pathogenic with varying degrees of aggressiveness at 14 days after inoculation (DAI). The most aggressive isolates were obtained in AEZ II (GA1, WA and MB with aggressiveness of 84; 84.33 and 76.67 % respectively), AEZ III (BAM, 84 %) and AEZ V (MF2, 79.33 %). These morphological characters and different degrees of aggressiveness reveal a great variability of *P. pachyrhizi* uredospores in Cameroon.

**Keywords**: aggressiveness, Asian rust, *Glycine max* L., phenotypic characterization.

## INTRODUCTION

*Phakopsora pachyrhizi* Sydow & Syd is the fungus responsible for Asian rust, which is one of the most dreaded diseases of soybean (*Glycine max* L.) in the world (Ibiam *et al.,* 2014). In Africa and in Cameroon in particular, *P. pachyrhizi* constitutes a major constraint to soybean production. Belonging to the large class of Basidiomycetes, family Phakopsoraceae (Ono *et al.,* 1992), *P. pachyrhizi* infects 95 species in 42 legume genera in the natural conditions (Hartman and Wang, 1992). *P. pachyrhizi* grows at temperatures between 15 and 28°C. It is a biotrophic fungus that grows and fruits only on living plant fragments (Blum *et al.,* 2015). Uredospores of *P. pachyrhizi* move readily during the rainy season and high humidity periods (Hartman *et al.,* 2011). *P. pachyrhizi* attacks the aerial part of the plant resulting in reduced green leaf area, decreased photosynthetic activity, premature defoliation and immature seeds (Kumudini *et al.,* 2008). Huge losses are then estimated to be between 10 and 90% in mixed cropping, between 10 and 100% in monoculture for Africa in general and 80% for Cameroon in particular (Hartman *et al.,* 1991; Akinsanmi *et al.,* 2001). It was first observed in Japan in 1902 and since then has been found in most soybean producing countries (Hartman *et al.,* 2011; Murithi *et al.,* 2015). This pathogen was observed in Cameroon in 2005 (Levy, 2005).

Studies on the characterization of *P. pachyrhizi* found the variability in virulence depending on their reaction on different hosts (Bonde *et al.,* 2006; Miles

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*et al.,* 2006; Pham *et al.,* 2009). The aggressiveness of *P. pachyrhizi* population for a given geographic locality depends on the isolates (Murithi *et al.,* 2017). Indeed, Lin. (1966) describing six isolates of *P. pachyrhizi* in Taiwan demonstrated that virulence depends of isolate. In the world, a variability of *P. pachyrhizi* isolates have been described including 18 isolates in Japan (Yamaoka *et al.,* 2002, 2014); 3 isolates in Brazil (Yamanaka *et al.,* 2010) and the United States of America (Twizeyimana and Hartmann, 2012). In Africa 17 isolates have been described with the most virulent isolate obtained in South Africa (Murithi *et al.,* 2017). Seven resistance genes to *P. pachyrhizi* have been identified in soybean (Rpp1, Rpp2, Rpp3, Rpp4, Rpp5, Rpp6 and Rpp7) and known to confer resistance to specific isolates of the pathogen (Garcia *et al.,* 2008; Li *et al.,* 2012). However, new pathotypes of *P. pachyrhizi* are emerging due to selection pressure. In Cameroon, very little information regarding the characterization of *P. pachyrhizi* isolates is available to date. The phenotypic characterization of *P. pachyrhizi* isolates at the local level remains therefore essential for the development of a rational and efficient control strategy (Schiffers and Moreira, 2011; Cooke *et al.,* 2012). The hypothesis tested in this work is that the knowledge of the virulence of macroscopic and morphometric forms of *P. pachyrhizi* pathotypes varies from one agro-ecological zone to another. The objective of this study is to describe the phenotypic characters of some isolates of *P. pachyrhizi* in Cameroon.

## MATERIALS AND METHODS

**Study area:** Samples were collected at 23 sites in the five agro-ecological (AEZ) zones of Cameroon (Table 1). Where AEZ I corresponds to Sudano-Sahelian zone; AEZ II to Guinean high savannas; AEZ III to western highlands; AEZ IV to Humid forests with monomodal rainfall; AEZ V to Humid forests with bimodal rainfall. These coordinates were used to draw up a sampling map showing the sites by agro-ecological zone (Figure 1).

Table 1. Sampling sites in each agro-ecological zone (AEZ)

| AEZ | localities | Longitude | Latitude | Altitude |
|---|---|---|---|---|
| AEZ I | Tokobere | 14°8′39,876′′ | 10°52′7,338′′ | 443 m |
| | Mokolo | 13°51′41,88′′ | 10°43′15,846′′ | 779 m |
| | soukoudou | 13°51′56,502′′, | 9°50′34,776′′ | 378 m |
| | Sanguere | 13°27′27,366′′ | 9°16′16,404′′ | 225 m |
| | Kodec | 14°24′35,67′′ | 10°39′9,318′′ | 369 m |
| AEZ II | Mbe | 13°36′7,626′′ | 7°51′7,344′′ | 582 m |
| | Wack II | 13°33′7,53′′ | 7°41′16,158′′ | 669 m |
| | Ngaoundéré I | 13°60′4,97′′ | 7°34′7,63 | 1124 m |
| | Ngaoundéré II | 13°55′9,75′′ | 7°29′8,60′′ | 1124 m |
| | Ganganssoua | 13°46′18,522′′, | 7°31′50,346′′, | 1116 m |
| AEZ III | Bamenda | 10°8′32,892′′ | 5°56′43,656′′ | 1264 m |
| | Mbouda | 10°14′48,444′′ | 5°38′9,834′′ | 1416 m |
| | Bangangté | 10°32′15,33′′ | 5°8′42,462′′ | 12943 m |
| | Bafoussam | 10°26′44,154′′ | 5°27′19,884′′ | 1493 m |
| | Santchou | 9°59′8,304′′ | 5°14′53,178′′ | 719 m |
| AEZ IV | Lala | 9°48′2,810′′, | 4°79′7,739′′ | 143 m |
| | Pendja | 9°41′34,458 | 4°38′28,272′′, | 1700 m |
| AEZ V | Mfou | 11°37′39,06′′, | 3°45′26,664′′ | 699 m |
| | Mbalmayo | 11°30′48,756′′ | 3°32′37,02′′ | 670 m |
| | Bafia | 11°13′59,592′′ | 4°44′27,756′′ | 483 m |
| | Fourgerole | 11°33′42,114′′ | 3°54′39,66′′ | 694 m |
| | Batchenga | 11°41′28,842′′ | 4°17′11,82′′ | 508 m |
| | Nkolbisson | 11°27′28,15′′ | 3°52′10,56′′ | 732 m |

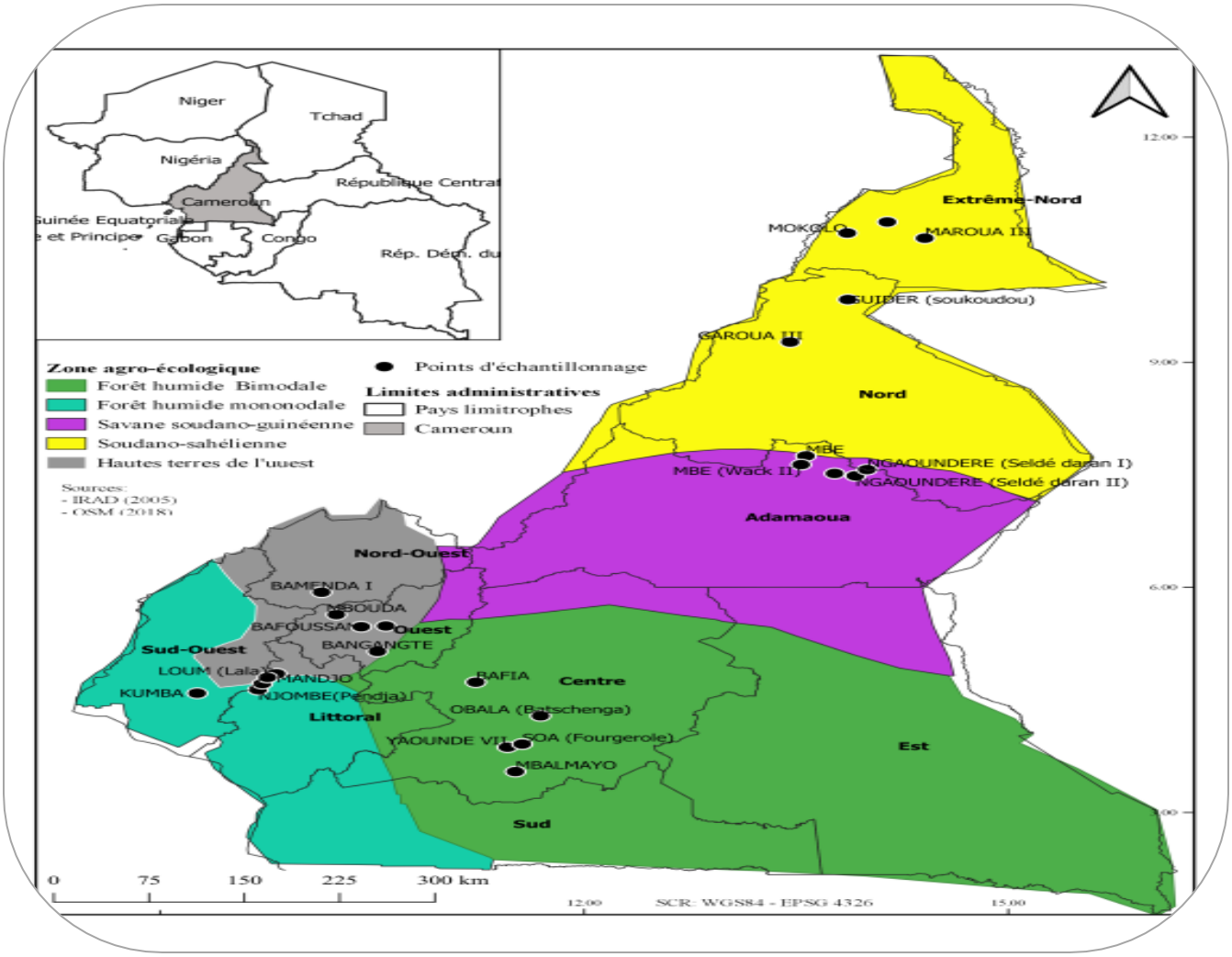


Figure 1. Map of Cameroon showing the sampling sites

**Collection of samples and naming of isolates:** Diseased plants were identified by visual observation of symptoms and fruiting bodies (pustules) on the lower part of diseased leaves (Ambang *et al.,* 2008; Renard and Foucart, 2008). These diseased leaflets were detached from the plant by pruning shears and inserted into envelopes, then transported to the laboratory. A total of 45 samples were collected from the five agro-ecological zones of Cameroon (Table 2). These samples were collected at a distance of more than 7 km from one site to another.

**Isolation of *Phakopsora pachyrhizi* isolates:** The pustules present on the lower parts of the diseased leaflet samples transported to the laboratory were carefully brushed separately with a fine brush into petri dishes to obtain isolates (Furtado *et al.,* 2008). These isolates were introduced into 20 ml of sterile distilled water. The suspensions of uredospore obtained from isolates were inoculated for multiplication on detached soybean leaflets phenotypically healthy, introduced into petri dishes containing a moistened filter. After 12 days, pure and fruiting cultures of *Phakopsora pachyrhizi* were again separately brushed and introduced into eppindrof tubes and stored in a refrigerator at -80˚C (Furtado *et al.,* 2008).

**Morphological and morphometric description of uredospore of *Phakopsora pachyrhizi* isolates:** The different isolates obtained were mounted with slide and coverslip. The morphologies of the different isolates according to the agro-ecological zone (AEZ) were obtained by microscopic observation. An optical microscope with a micrometer objective lens was used to measure the size of uredospore [length (L) and width (w)] at X 100 magnification. Then, the length/width ratio (L/w) was calculated for each AEZ (Duan *et al.,* 2008).

Table 2. Denomination and origins of P*hakopsora pachyrhizi* isolates

| AEZ | Localities | Isolates | Varieties | Collected organs |
|---|---|---|---|---|
| AEZ I | Tokobere | TO | TGX-1448-2E | leaves |
| | soukoudou | SO | TGX-1448-2E | leaves |
| | Sanguere | SA1 | R3 | leaves |
| | | SA2 | R2 | leaves |
| | | SA3 | PANAMA | leaves |
| | Mokolo | MO | TGX-1448-2E | leaves |
| AEZ II | NgaoundéreI | NGI | R2 | leaves |
| | NgaoundéreII | NGII | R2 | leaves |
| | Mbe | MB | R3 | leaves |
| | wack II | WA | TGX-1448-2E | leaves |
| | Ganganssoua | GA1 | TGX-1835-10E | leaves |
| | | GA2 | TGX-1485-1D | leaves |
| | | GA3 | TGX-1448-2E | leaves |
| AEZ III | Bangangté | BA1 | TGX-1835-10E | leaves |
| | | BA2 | LOCAL | leaves |
| | | BA3 | TGX-1835-10E | leaves |
| | Santchou | SAN | TGX-1835-10E | leaves |
| | Bamenda | BAM | TGX-1838-10E | leaves |
| | Diandam | DIA | TGX-1835-10E | leaves |
| | Mbouda | MBO | LOCAL | leaves |
| AEZ IV | LALA | LAL | LOCAL | leaves |
| | Pendja | PEN | LOCAL | leaves |
| AEZ V | Mbalmayo | MBA1 | TGX-1835-10E | leaves |
| | | MBA2 | TGX-1835-10E | leaves |
| | Nkolbisson | NKO | TGX-1991-22F | leaves |
| | Batchenga | BAT | TGX-1835-10E | leaves |
| | Bafia | BAF | TGX-1835-10E | leaves |
| | Mfou | MF1 | SCS-1 | leaves |
| | | MF2 | DPSB8 | leaves |
| | | MF3 | TGX-2014-19FM | leaves |
| | | MF4 | S1140-5-4 | leaves |
| | | MF5 | SOUNG PUNGU | leaves |
| | | MF6 | SNKGMO11 | leaves |
| | Fougerole | FOU | LOCAL | leaves |

**Evaluation of the aggressiveness of *Phakopsora pachyrhizi* isolates:** Detached leaflets of soybeans phenotypically healthy were collected in the field from the variety TGX-1835-10E from the Institute of Agricultural Research for Development in Foumbot. These leaves were washed three times with distilled water and then placed in 90 mm petri dishes containing a moistened filter. A suspension of uredospore with a concentration of 3 × $10^2$ Uredospores/ml per isolates previously calibrated using Malacez cell, was deposited on the detached leaflets of the visible part (abaxial) in Petri dish using a micropipette at a rate of three drops of 20µl per leaflet. Three replicates were performed per isolate given a total of 99 petri dishes. The aggressiveness of the isolates was assessed by three-day intervals according to the scale of 1 to 5 previously defined from that established by Godoy *et al.* (2006), where:

1- Percentage varies from 1 to 20%; there is formation of pustules without appearance of lesions;

2- Percentage varies from 21 to 40%; appearance of pustules and lesions;
3- Percentage varies from 41 to 60%; appearance of pustules, lesions and yellowing around the pustules;
4- Percentage varies from 61 to 80%; appearance of pustules, lesions and formation of blackish spots around the pustules;
5- Percentage varies from 81 to 100%; total necrosis of the leaf

**STATISTICAL ANALYSIS**

The data obtained from the morphometric and aggressiveness of the different isolates were subjected to an analysis of variance (ANOVA) using the R software version 3.5.1 (R Development Core Team 2022). The difference between the various means was compared using the Duncan's test when normality test (Shapiro-Wilk test $P > 0.05$) and homogeneity (Levene's test $P > 0.05$) of variance were verified. When the data did not follow a normal distribution, the Kruskal-Wallis test was used ($P < 0.05$).

**RESULTS**

**Identified Pathogen and number of isolates collected:** The technique of isolating and multiplying from pustules developed on detached leaflets in 12-day-old petri dishes yielded 34 isolates. The macroscopic (Figure 2A.) and microscopic (Figure 2B, 2C, 2D and 2E.) characteristics of the 34 isolates from the five agro-ecological zones were observed in the laboratory. The fungal fruiting bodies or uredinia (pustules), aged of 12 days in culture, were whitish and changed to brownish with age. The fungal fruiting bodies or uredinia developed in a solitary or grouped manner on the lesions and most of them were scattered on the lower part of the leaflets. Observed under a microscope (magnification ×100), these mature uredinia released uredospores (Figure 2B.). These uredinia by germination (Figure 2D.) and thanks to their specialized appressorium structure which are typical characteristics of *P. pachyrhizi*, will be able to infect other plants. The structure of teliospore have also been observed (Figure 2E).

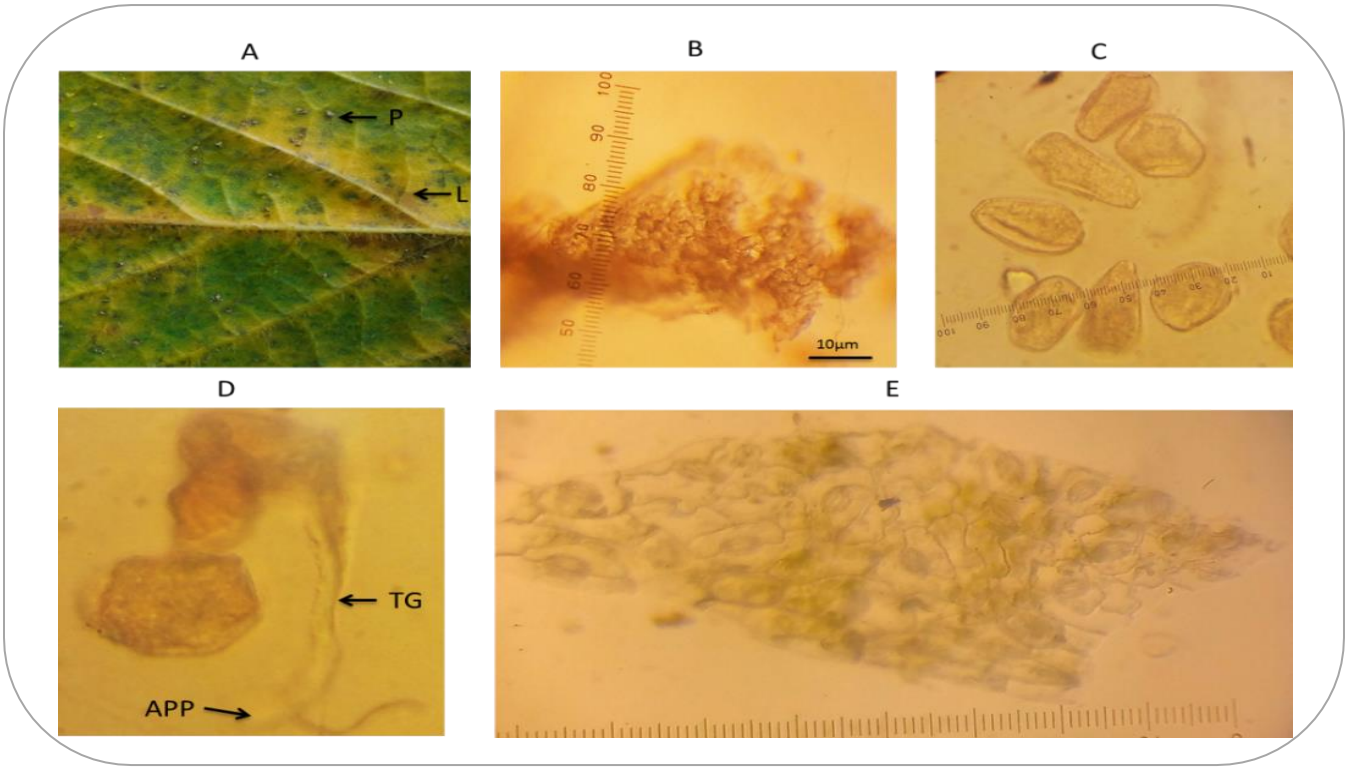


Figure 2. Macroscopic and microscopic characteristics of a pure culture of *Phakopsora pachyrhizi* isolates seen at X 100 magnification. (A) uredinia, (B) release of urediniospores, (C) urediniospores, (D) germination of uredospores (TG. germ tube, APP. appressorium), (E) teliospore structure.

**Morphologies of uredospores:** The forms of uredospores were much diversified. They varied very little according to localities and agro-ecological zones. A total of 07 forms were identified. Namely: the pepiform, elliptical, sub-spherical, amydaliform, globose, angular and sub-angular forms. These forms obtained, show a great variability of uredospores in Cameroon with the presence of two new forms; the seed-shaped (Figure 3B.) and angular (Figure 3F.).

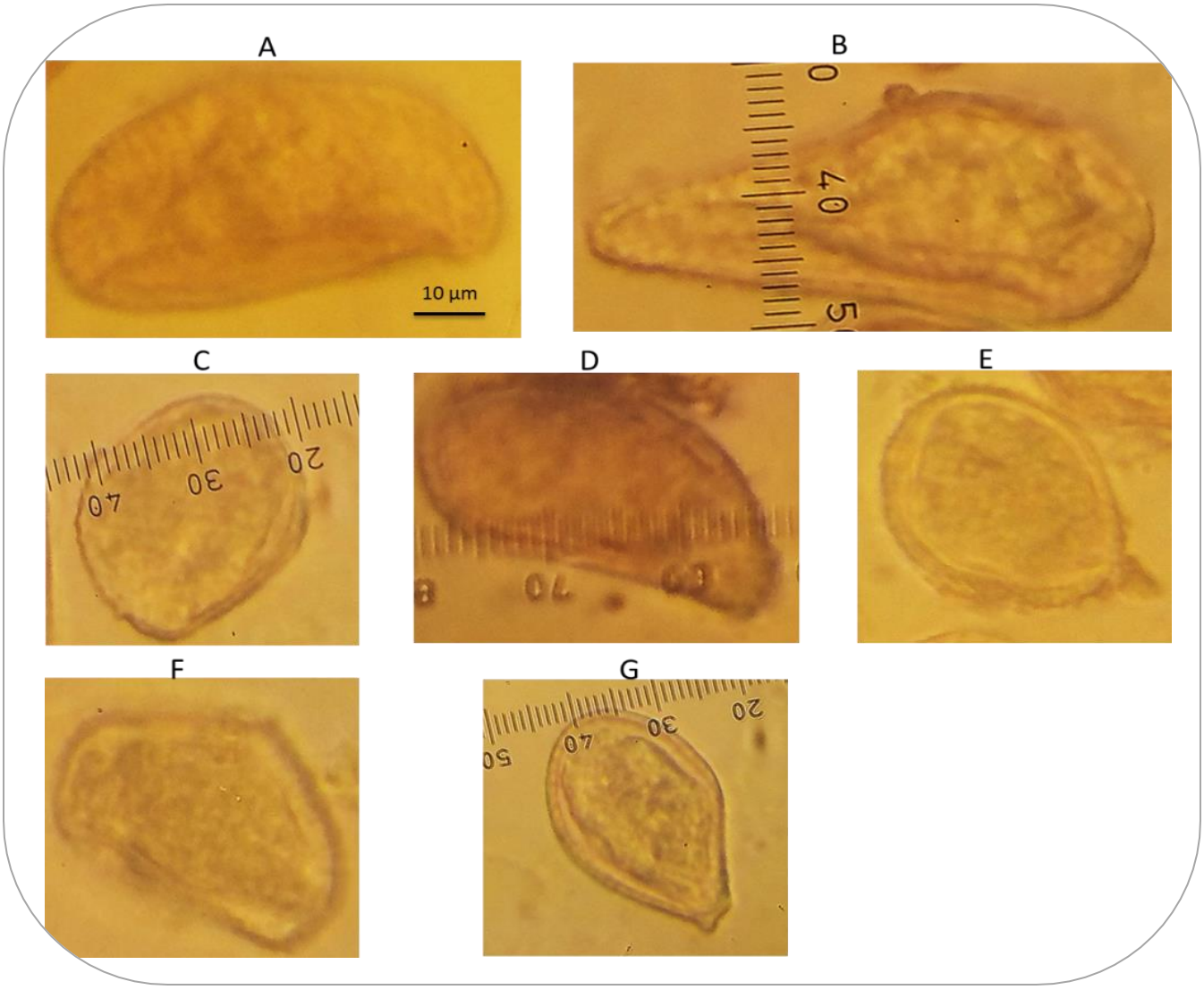


Figure 3. Forms of uredospores of *Phakopsora pachyrhizi* isolated in the five agro-ecological zones of Cameroon observed at X 100 magnification. (A) elliptical, (B) pepiniform, (C) sub-spherical, (D) amydaliform, (E) globose, (F) angular (G) sub-angular

**Morphometries of the uredospores of *Phakopsora pachyrhizi:*** The parameters lengths, widths, and ratio (L/w) of *P. pachyrhizi* uredospores did not differ significantly $P < 0.05$ between isolates from localities in AEZ I and between isolates from localities in AEZ IV. Uredospore lengths ranged from 23.92 to 28.08 µm; 24.96 to 27.04 µm and widths from 15.6 to 18.72 µm; 15.6 to 17.68 µm for AEZ I and IV respectively. Their ratios (L/w) ranged from 1.42 to 1.72 and from 1.57 to 1.6 respectively. A significant difference is recorded between isolates from AEZ II localities, between isolates from AEZ III localities and between isolates from AEZ V localities in terms of uredospores lengths and widths. AEZ lengths ranged from 26 to 35.6 µm; 26 to 29.12 µm; 26 to 36.40 µm and widths from 17.68 to 22.88 µm; 15.60 to 19.76 µm; 15.60 to 21.84 µm respectively. The ratios (L/w) did not differ significantly between isolates from locations in AEZ II and III, but varied significantly between isolates from locations in AEZ V. These ratios (L/w) ranged from 1.33 to 1.55; 1.28 to 1.58 and 1.25 to 2.08 respectively. The isolate from Mbalmayo1 has the highest L/w ratio of 2.08. In general, there is no significant difference between the lengths or L/w ratio according to the AEZ, but there are significant differences between the widths of AEZ II and the other AEZ. These lengths vary from 25.40 to 27.64 µm; widths from 16.64 to 19.61 µm and L/w ratio from 1.45 to 1.57 (Table 3).

Table 3. Morphometry of uredospores of *Phakopsora pachyrhizi* isolates

| AEZ | localities | Uredospore lengths (L) in µm | Uredospore width (l) in µm | Ratio (L/l) |
|---|---|---|---|---|
| AEZ I | Tokobere | 23.92 ± 5.92a | 15.60 ± 3.67a | 1.68 ± 0.82a |
| | soukoudou | 28.08 ± 2.84a | 17.68 ± 2.84a | 1.63 ± 0.37a |
| | Sanguere 1 | 26.00 ± 5.2a | 18.72 ± 2.84a | 1.42 ± 0.37a |
| | Mokolo | 26.00 ± 4.24a | 18.20 ± 3a | 1.48 ± 0.19a |
| | Sanguere 2 | 28.08 ± 2.84a | 18.72 ± 2.84a | 1.53 ± 0.31a |
| | Sanguere 3 | 28.08 ± 2.84a | 16.64 ± 2.32a | 1.72 ± 0.30a |
| Average | | 26.69 ± 1.69a | 17.30 ± 1.06b | 1.57 ± 0.11a |
| AEZ II | Ngaoundére 1 | 27.04 ± 4.35 b | 22.88 ± 2.84a | 1.19 ± 0.20a |
| | Ngaoundére 2 | 28.08 ± 2.84b | 18.72 ± 2.84ab | 1.53 ± 0.31a |
| | Mbe | 22.88 ± 2.84b | 17.68 ± 2.84b | 1.33 ± 0.33a |
| | wack 2 | 27.04 ± 4.35b | 18.72 ± 5.92ab | 1.55 ± 0.44a |
| | Ganganssoua 1 | 35.36 ± 2.32a | 22.88 ± 2.84a | 1.56 ± 0.17a |
| | Ganganssoua 2 | 26.00 ± 5.2b | 17.68 ± 2.84b | 1.50 ± 37a |
| | Ganganssoua 3 | 29.12 ± 6.97b | 18.72 ± 2.84ab | 1.55 ± 0.24a |
| Average | | 27.21 ± 2.40a | 19.61 ± 2.28a | 1.45 ± 0.14a |
| AEZ III | Banganté 1 | 27.04 ± 4.35a | 17.68 ± 2.84ab | 1.57 ± 0.39 a |
| | Banganté 2 | 21.84 ± 2.32b | 15.60 ± 0.00b | 1.40 ± 0.14 a |
| | Banganté 3 | 26.00 ± 0.0a | 17.68 ± 2.84ab | 1.50 ± 0.22 a |
| | Santchou | 27.04 ± 2.32a | 17.68 ± 2.84ab | 1.55 ± 0.18a |
| | Bamenda | 26.00 ± 3.67a | 16.64 ± 2.32ab | 1.58 ± 0.3a |
| | Diandam | 20.80 ± 3.67b | 16.64 ± 2.32ab | 1.28 ± 0.33a |
| | Mbouda | 29.12 ± 2.84 | 19.76 ± 2.32a | 1.48 ± 0.14a |
| Average | | 25.40 ± 2.99a | 17.38 ± 1.30b | 1.48 ± 0.1a |
| AEZ IV | Lala | 27.04 ± 2.32a | 17.68 ± 2.84a | 1.57 ± 0.31a |
| | Pendja | 24.96 ± 4.35a | 15.60 ± 0.00a | 1.60 ± 0.27a |
| Average | | 26.00 ± 1.47a | 16.64 ± 1.47b | 1.58 ± 0.02a |
| AEZ V | Balmayo 1 | 36.40 ± 3.67a | 17.68 ± 2.84abc | 2.08 ± 0.25a |
| | Mbalmayo 2 | 20.80 ± 3.67c | 16.64 ± 2.32bc | 1.25 ± 0.14c |
| | Nkolbisson | 27.04 ± 2.32b | 18.72 ± 2.84abc | 1.50 ± 0.20bc |
| | Batchenga | 28.08 ± 2.84b | 15.60 ± 0.00c | 1.80 ± 0.18ab |
| | Bafia | 26.00 ± 3.67b | 18.72 ± 2.84abc | 1.42 ± 0.28bc |
| | Mfou 1 | 35.36 ± 4.35a | 19.76 ± 2.32a | 1.82 ± 0.35ab |
| | Mfou 2 | 27.04 ± 2.32b | 21.84 ± 2.32abc | 1.25 ± 0.17c |
| | Mfou 3 | 27.04 ± 6.7b | 18.72 ± 2.84abc | 1.45 ± 0.29bc |
| | Mfou 4 | 28.08 ± 2.84b | 19.76 ± 4.35abc | 1.47 ± 0.34bc |
| | Mfou 5 | 26.00 ± 0.00b | 19.76 ± 2.32abc | 1.33 ± 0.18c |
| | Mfou 6 | 23.92 ± 2.84bc | 20.80 ± 3.67ab | 1.19 ± 0.32c |
| | Fougerole | 26.00 ± 3.67b | 18.72 ± 2.84abc | 1.43 ± 0.39bc |
| Average | | 27.64 ± 4.33a | 18.21 ± 1.04ab | 1.49 ± 0.26ab |

P: 0 '***' 0.001 '**' 0.01 '*' 0.05. Values in each column with the same letters are not significantly different based on Duncan's test of probability at *0.05*.

**Aggressiveness of *Phakopsora pachyrhizi* isolates on detached soybean leaves:** All the 34 isolates of *P. pachyrhizi* obtained induced disease on detached soybean leaflets in petri dishes. The uredin and lesions observed were typically those of Asian rust. The degree of aggressiveness varied among isolates at 3, 6, 9, and 14 days after inoculation (DAI). At 14 days after inoculation, the degree of aggressiveness of isolates ranged as follow:

GA1 (84 %), WA (84.33 %), MB (76.67 %) in AEZ II; BAM (84 %) in AEZ III and MF2 (79.33 %) in AEZ V showing the highest degree of aggressiveness. The lowest degrees of aggressiveness were obtained with isolates MO (46 %), TO (52 %) belonging to AEZ I and isolate BA1 (50 %) belonging to AEZ III (Table 4.).

Table 4. Aggressiveness of the different isolates during the different data collection

| Isolates | 3 JAI | 6 JAI | 9 JAI | 14 JAI |
|---|---|---|---|---|
| TO | 8.33 ± 0.57mno | 12.67 ± 1.15p | 19.67 ± 3.78r | 52 ± 6.24fgh |
| SO | 14.67 ± 1.15fgh | 19.67 ± 2.08op | 28.67 ± 1.52opqr | 59.33 ± 4.93ef |
| SA1 | 16 ± 1.0def | 27.33 ± 2.08ghij | 42.67 ± 3.21efg | 55.67 ± 3.21fg |
| SA2 | 13 ± 1.73hij | 26.67 ± 2.51hijk | 33.33 ± 5.13klmno | 53.33 ± 3.78fgh |
| SA3 | 10.67 ± 1.52jklm | 24.67 ± 3.21jklm | 38.67 ± 1.52ghi | 66.33 ± 7.37de |
| MO | 21.67 ± 1.52ab | 31.33 ± 2.51cdef | 43.67 ± 1.52ef | 46.33 ± 6.50h |
| NG1 | 11.33 ± 2.08jkl | 22.33 ± 2.30mnop | 31.33 ± 3.21nopq | 70.33 ± 2.08bcd |
| NG2 | 21.67 ± 1.15ab | 28.33 ± 2.08fghi | 40.± 3fgh | 72.33 ± 3.78bcd |
| MB | 19 ± 1bc | 35.67 ± 2.08abcd | 45.33 ± 3.05cde | 76.67 ± 3.05abc |
| WA | 12.33 ± 2.08ijk | 32.33 ± 2.51bcde | 46 ± 2.0bcde | 84.33 ± 5.13a |
| GA1 | 38.67 ± 1.15a | 50.33 ± 2.88a | 70 ± 3a | 84 ± 5.29a cd |
| GA2 | 5 ± 1.7o | 28.33 ± 1.52fghi | 36.67 ± 3.21hijkl | 70 ± 2cd |
| GA3 | 11 ± 1jklm | 29 ± 1.73efg | 35.33 ± 3.05ijklmn | 66 ± 7de. |
| BA1 | 8.33 ± 1.52mno | 21 ± 1.73nop | 38 ± 2.64hij | 50.67 ± 5.13bcd |
| BA2 | 17.67 ± 1.52cd | 38 ± 1.73abc | 44 ± 1.73def | 75 ± 2.64gh |
| BA3 | 15 ± 1efg | 40.67 ± 2.08ab | 50 ± 2.64abc | 73.67 ± 3.05bcd |
| SAN | 8.67 ± 1.52lmno | 25 ± 2jklm | 42.67 ± 2.08efg | 74.67 ± 2.30bcd |
| BAM | 14.67 ± 1.52fgh | 26 ± 1.73jkl | 37 ± 4.35hijkl. | 84 ± 5.56a |
| DIA | 14.33 ± 0.51ghi | 30.33 ± 1.52def | 38 ± 1.73hij | 73.33 ± 3.05bcd |
| MBO | 16.33 ± 1.52def | 28.67 ± 1.52fgh | 36.33 ± 2.08ijklm | 73.33 ± 2.51bcd |
| LAL | 16.67 ± 1.52cde | 24 ± 1.73lmno | 33.67 ± 1.52lmno | 67.67 ± 2.5cd |
| PEN | 24 ± 1a | 29.33 ± 1.52efg | 44.67 ± 2.51cde | 73.33 ± 4.16bcd |
| MBA1 | 17.67 ± 2.08cd | 25.67 ± 1.15jkl | 35.33 ± 1.52jklmn | 72.33 ± 7.50bcd |
| MBA2 | 12.67 ± 1.51ijk | 31 ± 2.64def | 38 ± 2.64hij | 70 ± 6.92cd |
| NKO | 6.33 ± 1.52o | 24.33 ± 2.08klmn | 35.33 ± 1.52 jklmn | 73 ± 3bcd |
| BAT | 17.67 ± 2.51cd | 22.33 ± 1ijkl | 26 ± 2pqr | 70.67 ± 1.52bcd |
| BAF | 17.67 ± 1.52cd | 26.33 ± 2.08mnop | 55 ± 3ab | 73.33 ± 2.88bcd |
| MF1 | 22.33 ± 2.08ab | 39.33 ± 3.05ab | 49.33 ± 3.05abcd | 74.33 ± 3.51bcd |
| MF2 | 10 ± 1.0klmn | 32.33 ± 2.30bcde | 47.33 ± 2.51abcde | 79.33 ± 2.51ab |
| MF3 | 14 ± 1ghi | 25.67 ± 1.52jkl | 37.33 ± 3.05hijk | 71 ± 2bcd |
| MF4 | 31.67 ± 1.52a | 41.33 ± 3.05ab | 50.67 ± 4.16abc | 66.33 ± 8.96 de |
| MF5 | 14.33 ± 1.52ghi | 25 ± 2klm | 32.33 ± 2.30mnop | 71.33 ± 4.16bcd |
| MF6 | 17.33 ± 2.51cd | 26.33 ± 1.52ijkl | 32.67 ± 2.08mnop | 71 ± 3.60bcd |
| FOU | 7.67 ± 1.15no | 17.33 ± 2.08p | 24 ± 2.64qr | 71.67 ± 3.05bcd |

P: 0 '***' 0.001 '**' 0.01 '*' 0.05. Values in each column with the same letters are not significantly different based on the Kruskal-wallis and Duncan's test of probability (*P < 0.05*).

**DISCUSSION**

The phenotypic characterization and identification of *P. pachyrhizi* is necessary and essential for the development of a rational and effective control strategy (Bruno and Christine, 2011; Cooke *et al.,* 2012). This study reports the lack of information about the pathogen at the local level and therefore the phenotypic characterization would present variants related to the isolates from the different AEZ. The results of the macroscopic and morphometric characterization of *P. pachyrhizi* isolates show a variability of morphological characters. Indeed, Bonde and Brown. (1980); Roxana *et*

*al.* (2016) working respectively on the morphological comparison of *P. pachyrhizi* isolates from different regions of the world and on the germination and infection of *P. pachyrhizi* under simulated environment of the central zone in Argentina obtained morphologies that vary from ovoid to ellipsoid.

In the present work, a total of 7 forms were identified, with the appearance of two new forms of uredospores of *P. pachyrhizi* in the different agro-ecological zones in Cameroun. The forms obtained show a great variability of *P. pachyrhizi* uredospores. Sachin *et al.* (2020) observed and identified three forms of *P. pachyrhizi* uredospores in different localities of Karnataka in India. Overall, the sizes of uredospores obtained from the AEZ showed no significant differences. Nevertheless, some significant differences exist between the widths. The lengths vary from 25.40 to 27.64 µm; the widths from 16.64 to 19.61 µm and the ratio (L/w) from 1.45 to 1.57. The variations obtained could be due to the variability of the shapes present (Dida *et al.,* 2020). These results are in agreement with the work of Ono *et al.* (1992) who showed that the lengths of uredospores varied from (18 to 34 µm) and widths (15 to 24 µm). On the other hand, Igarashi *et al.* (2012) working on the comparative morphology between uredospores of agriculturally important species obtained lengths and widths ranging from 26 to 39 and 19 to 25 respectively.

*P. pachyrhizi* isolates obtained from the different AEZ were all found to be pathogenic with variability in aggressiveness among isolates. Isolates GA1, WA, MB, BAM and MF2 were more aggressive compared to isolates MO, TO and BA1. The variability in aggressiveness among isolates may be due to variability in virulence genes or to the fusion of germ tubes into germinating uredospores, creating a complex hyphae network, into which nuclei migrate to form the multi-nucleate hyphae as demonstrated by Vittal *et al*. (2012). Indeed, these authors demonstrated that the anastomosis and the formation of heterokaryons would be at the origin of the genetic variability of virulence genes in *P. pachyrhizi* populations. The variability of aggressiveness may also be a function of their reaction on different hosts as stated by Bonde *et al.* (2006); Miles *et al.* (2006) and Pham *et al.* (2009).

**CONCLUSION**

The general objective of this study was to characterize phenotypically the isolates of *P. pachyrhizi* in Cameroon. The results show a significant variation in the morphological characters of *P. pachyrhizi* uredospores. Seven forms were observed with the appearance of two new forms. The pepiniforms and angular forms. These forms obtained show a great variability of uredospores of *P. pachyrhizi* in Cameroon. The lengths and ratio (L/w) of uredospores did not differ significantly between AEZ. However, there were significant differences between the widths of AEZ II and the other AEZ. The lengths varied from 25.40 to 27.64; the widths from 16.64 to 19.61 and the ratio (L/w) from 1.45 to 1.57. All 34 *P. pachyrhizi* isolates obtained were pathogenic with a degree of aggressively varying from an isolate to another at 14 days after inoculation. The most aggressive isolates were obtained in AEZ II (GA1, WA, and MB), AEZ III (BAM) and AEZ V (MF2).

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**CONTRIBUTION OF AUTHORS**

Zachée Ambang, selected the scope of the work and reviews the manuscript. Fabrice Christian Gbaporo Gbaporo conduct the Lab experiments and writes the first draft of the manuscript. Patrice Zemko Ngatsi analyses data and review the manuscript. Sylvère Landry Lontsi Dida and Cédrik B. W. Chedjou, conduct manipulation in lab. Angèle Pégalepo Ndogho and Sylvère Landry Lontsi Dida read and review the manuscript. All authors have read and approved the final version of the manuscript.

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|---|---|
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| Patrice Z. Ngatsi | : Analyses data and review the manuscript data and review de manuscript |
| Cédrik B. W. Chedjou | : Conduct manipulation in lab |
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