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RESEARCH ARTICLE

MORPHOLOGICAL VARIABILITY WITHIN POPULATIONS OF *ALTERNARIA PORRI* (ELLIS) CIF., THE CAUSATIVE AGENT OF ONION PURPLE BLOTCH DISEASE IN BURKINA FASO

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Abstract

Onion production in Burkina Faso remains limited due to certain devastating diseases such as purple blotch caused by *Alternaria porri*. This disease causes significant yield losses and is difficult to control with chemicals. This difficulty could be linked to genetic, pathogenic and/or pathogenic variability within populations of *A. porri*. The objective of the present study was to evaluate the morphological variability of *Alternaria porri* populations occurring in Burkina Faso with a view to better define appropriate methods in controlling the disease. Thus, fifty isolates of *A. porri*, obtained from diseased onion leaves collected in the different agroclimatic zones, were morphologically characterized. Multivariate analyses were carried out on the data collected using XLSTAT 2016.7.02 software. These analyses revealed variability of *A. porri* in its morphological aspects such as colony color, radial growth and micropropagules production. All of the characterized isolates were grouped into three classes. Class 1 with 34 individuals, characterized by weak radial growth but high production of micro propagules. The second class composed of four isolates, is characterized by individuals with strong mycelial growth and a low capacity for the production of micro propagules. A third class, composed of twelve isolates, is characterized by intermediate individuals in terms of growth and production of micro propagules. However, this clustering into classes did not align with the geographic origin of the isolates. A pathogenicity study should be carried out with representatives of the three classes in order to

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understand whether this observed morphological variability is linked to the virulence of the fungus.

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..... Introduction:-

In Burkina Faso, market gardening makes a significant contribution to reducing poverty and unemployment in rural areas (MAH/DGPSAA, 2011; Oboulbiga et al., 2023). This agricultural sector, which has been listed as a priority in the country's agricultural policies since 2007, generates around 600,000 permanent jobs and contributes around 5% to the gross domestic product (GDP) (MAHRH, 2007). Market gardening in Burkina Faso presents unprecedented investment opportunities with the availability of 233,500 ha of irrigable land, 1,700 surface water points and abundant and cheap labor (Bambio, 2021).

Among the produced and marketed vegetables, onion (*Allium cepa* L.) ranks first in terms of allocated areas and corresponding produced quantity (MAH/DGPSAA, 2011; MASA, 2013). According to recent statistics, Burkina Faso is the second largest onion exporting country in West Africa after Niger (Bambio, 2021). Onion sub-sector has been booming in recent years in Burkina due to its high profitability and its great capacity to generate jobs for young people and women. Onion generates an average farm income per hectare of 8 855 500 XOF (around 15,000 USD) (Yili, 2013).

Onion production has risen from 242,258 tons in 2008 to 362,480 tons in 2018, an increase of around 50% (Bambio, 2021). However, this increase is linked to the increase in areas because average yields decreased from 20T/ha to 19T/ha the same decade (MAAHM, 2021). These yield levels remain very low compared to the potential yields of onion varieties ranging from 40 to 60 T/ha (Guissou et al., 2012; CNUCED, 2014; Kintega et al., 2020).

The low yields observed are due to several causes, among which the infection by plant pathogens (Boukary, 2014). These plant pathogens occurred at all stages of plant development and cause significant pre- and post-harvest losses (McCallum, 2007; Boukary, 2014). In the world, more than 75% of onion diseases are caused by fungi (Schwartz et Mohan, 2008; Rabiei-Motlagh et al., 2010; Ghanbarzadeh et al., 2014). In Burkina Faso, onion purple blotch disease, caused by *Alternaria porri* (Ellis) Cif., is the most prevalent and widespread fungal disease (Dabiré, 2017). It is a foliar disease (Figure 1) showing small purple circular necrotic spots on the leaves. These small spots rapidly enlarge and join together resulting in a complete drying of the infected leaf. In high humidity, temperature and planting density conditions, the infection can lead to complete drying of the plant all over the field (Prakasam, 2010; Maldhavi et al., 2012).

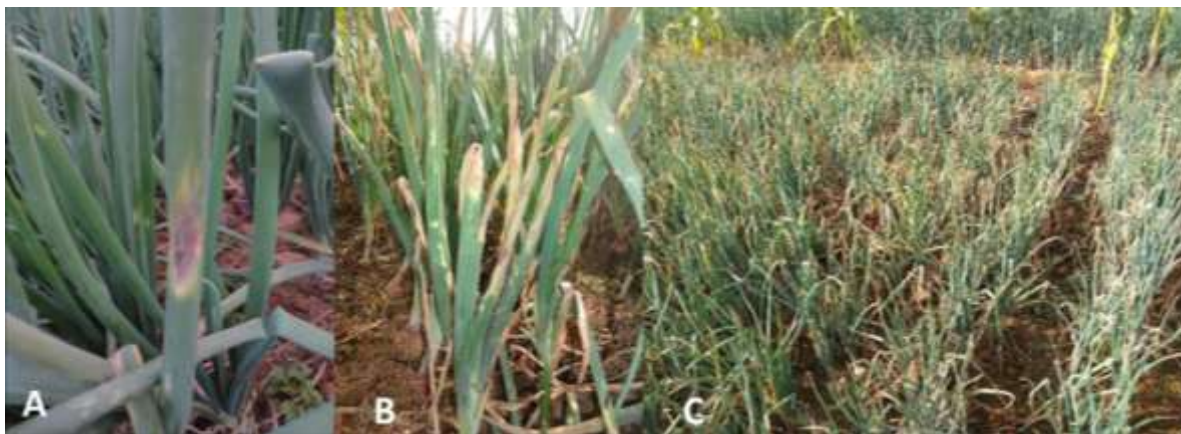


Figure 1:- Purple blotch evolution. (A): Necrotic spot; B: Symptoms on onion plant; (C): Advanced field infection

Purple spot disease causes heavy yield losses in the field and bulb rot during storage (Chethana et al., 2018). In India, it is reported that this disease can cause more than 60% yield loss (Shahanaz et al., 2007; Vinny et al., 2018). Consequently, several onion production sites have been abandoned due to the strong presence of this disease (Dabiré, 2017). Purple blotch disease poses a serious threat to the sustainability of Burkina Faso's onion sub-sector as high humidity, hot temperature and dense planting, all favorable to the fungus, are also common conditions in the

country (Prakasam, 2010; Omprakash, 2016). Purple blotch is difficult to control using current control methods. Rovral 50 WP (0.2%), Dithane M-45 80 WP (0.2) and some other fungicides are, in fact, suggested as foliar spraying against the disease. Yet, very rapidly, these fungicides became inefficient (Mohsin et al., 2016b). This might be due to the genetic variability or the development of new races of the pathogen. In addition to the negative impact on yields, *Alternaria porri* produces mycotoxins in onion leaves whose consumption constitutes a health risk to man and farm animals. This risk is high in Burkina Faso because onion leaves are processed and/or consumed raw by local populations (Tarpaga, 2012).

The symptoms observed in the field present a certain variability in their forms and their evolution according to the production zones. The severity of the disease as well as the success of phytosanitary treatments also vary depending on the production areas. The question then arises as to whether the variability of symptoms is linked to morphological variability within the populations of the pathogen. Understanding the pathogen population structures and the mechanisms by which variation arises within a population is of paramount importance for designing a successful disease management strategy (Mohsin et al., 2016a). Studies of variability within the populations of a plant pathogen is an important step in the search for appropriate and suitable methods for the management of the disease. It helps to understand the variability in the pathogenesis and geographical distribution of pathotypes (Priya et al., 2018). It can also lead to gathering basic information to understand the emergence of resistance to pesticides and also to developing epidemiological tools for forecasting epidemics (Mohsin et al., 2016b).

In Burkina Faso, no knowledge on the morphological, genetic and pathogenic variability of *A. porri* populations is available while the existence of variability within a pathogen could indicate the existence of pathotypes. We therefore intended to study the morphological variability of *A. porri* populations present in Burkina Faso in order to contribute to the development of an appropriate strategy for the management of purple blotch disease in the country.

Material and Methods: -

Diseased plant samples and sampling process

One hundred and sixteen (116) primary samples of onion plants whose leaves show typical symptoms of purple blotch disease were collected from various market gardening areas throughout the country. The territory of Burkina Faso is divided into three agro-climatic zones. A Sahelian zone characterized by an annual rainfall less than or equal to 600 mm, a North-Sudanian zone characterized by an annual rainfall between 600 and 900 mm and a South-Sudanian zone characterized by an annual rainfall greater than or equal to 900 mm. The choice of collection sites was made taking into account this agro-climatic zoning but also resorting to other additional criteria such as the scale of onion production and the accessibility of the sites. Thus, a total of 11 provinces and 22 villages were visited for the collection of these samples (Table 1). Information was previously sent to the producers of the various sites to be visited to identify the plots attacked by the disease. Once on the site, diseased plants were completely removed and put in Kraft paper envelopes. The identity of the producers, their contacts numbers, the date of collection and other additional information were then written on the envelopes in order to facilitate the traceability of each sample (Figure 2). The envelopes were then coded and stored in a cooler. Some prophylactic advice were finally given to the producers before leaving the site. Once in the laboratory, a second sampling was performed on the primary samples from each site through closer inspection of symptoms. This inspection made it possible to retain 50 secondary samples presenting more typical symptoms of the disease. The isolation of *A. porri* was carried out on these 50 primary samples.

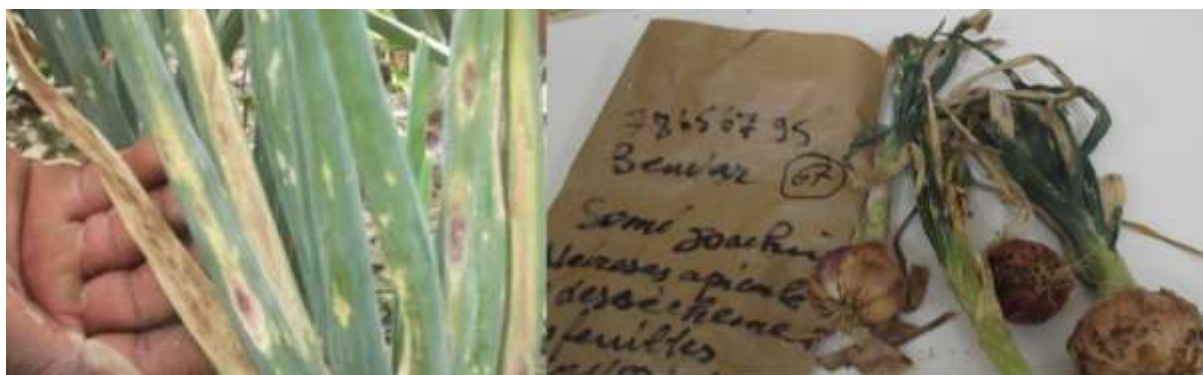


Figure 2: - Typical symptoms and sampling process.

Table 1:- Geographical distribution of collected samples.

Agro-climatic zones	Provinces	Villages/sites	Number of samples	
			primary	secondary
Sahelian zone	Yatenga	Tougou	5	2
		Toessin	5	2
		Goinré	6	2
		Touzagouè	5	2
	Sanmatenga	Korsimoro	5	2
North-Sudanian zone	Sourou	Di	17	10
		Niassan	5	2
		Dèbè	5	2
		Gouran	5	2
	Banwa	Gnanssoumadougou	5	2
		Siwiktou	5	2
	Kadiogo	Ouagadougou/Art3	1	1
		Ouagadougou/Art4	4	1
	Ganzourgou	Mogtèdo	5	2
	Oubritenga	Loumbila	5	2
South-Sudanian zone	Léraba	Douna	5	2
		Niantonon	5	2
	Houet	Soumousso	6	2
		Fô	5	2
		Bambé	5	2
	Bougouriba	Bapla	3	1
	Ioba	Benvar	4	3
	Total	11	22	50

Isolation and morphological identification of *A. porri*

The 'wet chamber' method as described by Blancardet al., (2000) was used for the isolation of *A. porri*. This method consists in placing the lesions of the diseased leaves in a humid atmosphere in order to promote the formation of conidia of the fungus on these lesions. On each sample, leaf fragments 2-3 cm long showing the typical lesions of the disease were then cut out, washed three times with sterile distilled water and then dried under a laminar flow hood (Figure 2A). After drying, 5 fragments were placed in a Petri dish lined with a triple layer of blotter papers moistened with sterile distilled water (Figure 2B). For each sample, 5 Petri dishes were used. The Petri dishes containing the fragments were then incubated at 25°C under near UV light alternated with darkness (12h/12) for three (03) days. At the end of the three (03) days of incubation, the incubated fragments were examined under a stereomicroscope at 16X and 25X magnifications to detect the presence of fungal colonies and identify *A. porri*. based on the morphological characteristics of the conidia (Figure 2C). When direct identification is doubtful under stereomicroscope, slides of the fungal colony were prepared and observed under an optical microscope at 10X and 40X magnifications to confirm the identity of the fungus.

Colony purification

A culture medium for fungi was prepared by dissolving 42 g of Potato Dextrose Agar (PDA) in 100 ml of sterile distilled water. The resulting solution was sterilized in an autoclave at 120°C for 30 minutes. After cooling, 0.25 g of streptomycin was added to the solution which was then homogenized and distributed in new Petri dishes. The conidia of *A. porri* formed on the fragments of incubated leaves were removed using a lanceolate needle and then cultured in the Petri dishes containing the PDA medium. The Petri dishes thus inoculated were incubated at 25°C under an alternating cycle of near ultraviolet light and darkness (12h/12h) for 7 days. From the colonies obtained at the end of the incubation time, a second subculture was carried out by taking small pieces of mycelium at the margin of the colonies and depositing them in new Petri dishes which were incubated under the same conditions as previously done. Pure isolates (Figure 3D) were thus obtained and stored in a freezer with the same codes as those of the original samples.

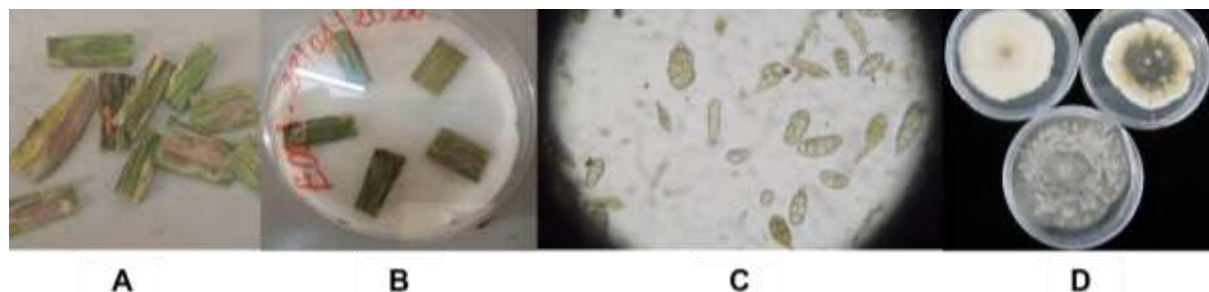


Figure 3: - Isolation, identification and purification of *A. porri* isolates from infected leaves. (3A): Diseased leaf fragments; (3B) Wet chamber method; (3C) Conidia of *A. porri* observed under optical microscope (400X); (D) Isolates of *A. porri* purified on PDA medium.

Assessment of the morphological variability of the isolates

The evaluation of the morphological variability within the populations of *A. porri* was carried out by considering quantitative and qualitative variables. The quantitative variables considered were (i) the radial growth, (ii) the number of circular bands of the mycelium and (iii) the production of micro propagules. The qualitative variables were (i) the color of the mycelium and (ii) the shape of the mycelial growth margins.

Quantitative variables

Radial growth

The radial growth is the average growth diameter of the fungus on the culture medium. Each isolate was cultured in PDA medium for 5 days. Using a 5 mm diameter cookie cutter, explants were removed and placed in the center of a Petri dish still containing the PDA culture medium under aseptic conditions. Petri dishes were sealed with parafilm paper and incubated at 25°C under 12 hours of near UV light alternated with 12 hours of darkness for 10 days. Two perpendicular straight lines were drawn on the back of the Petri dish passing through the center and the growth diameters were measured on each straight line using a slat at the 4th, 7th and 10th days after incubation (DAI) (Figure 4). For each isolate, four Petri dishes were evaluated, each Petri dish constituting a repetition. The mean growth diameters obtained from the measurements made on the two perpendicular straight lines were used as quantitative data.

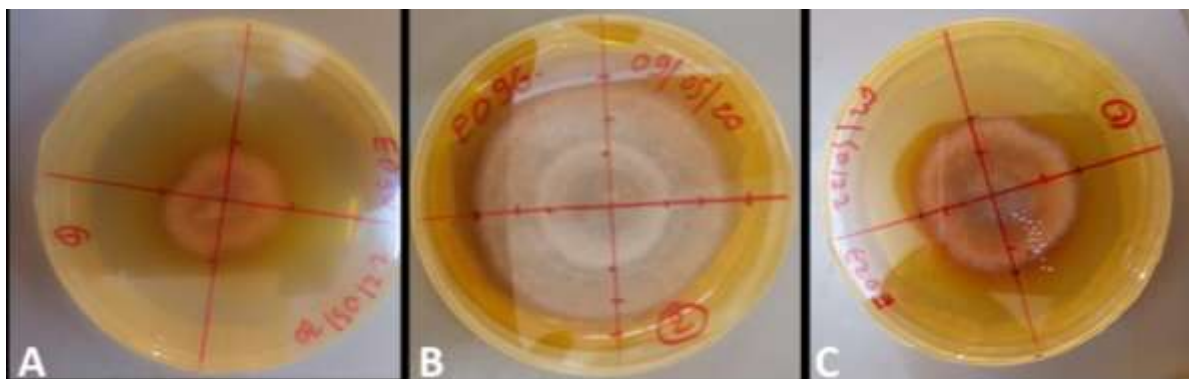


Figure 4:- Radial growth evaluation. (4A): 4 DAI; (4B): 10 DAI; (4C): 7 DAI

The number of circular bands formed

Alternaria porri grows on the PDA medium in concentric bands, the number of which changes over time. The number of these bands varies according to the isolates. For each isolate, the number of circular bands was noted after direct observation of the cultures 10 days after incubation.

Micro propagules production

For the micro propagules production, each isolate was cultured on PDA medium in four (04) repetitions then incubated at 25°C for 10 days. At the end of the culture period, the contents of each Petri dish were moistened with 10 mL of distilled water supplemented with one drop of Tween 80 (0.1%). The content was then homogenized by vortexing for three (03) minutes to obtain a mixture of conidia and mycelial fragments. This mixture was then diluted

10,000 times. Five hundred (500) µl of each aliquot were spread in a Petri dish containing the PDA culture medium which was then incubated at 25° C. for two days. Colonies grown in the Petri dish were counted visually (Figure 5). The results obtained were brought back to the initial concentration then expressed in Colony Forming Units per 500 mL (CFU/mL).



Figure 5:- Micro propagules assesment process. (5A): Serial dilution of the mycelial suspension ; (5B): Spreading aliquots on culture medium; (5C): Colony forming units after two (2) DAI

Qualitative variables

The qualitative variables such as the color of the mycelium and the shape of the steps were assessed by direct observation of four pure colonies of each isolate, after 10 days of culture.

Data analysis

The qualitative data collected were analyzed using Excel 2016 software. This analysis consisted in a simple calculation of the means and frequencies of each modality identified within the qualitative variables. The quantitative data collected was entered and organized with Excel 2016 software. Descriptive and multifactorial statistical analyses were then carried out using the XLSTAT software. Variability within isolates was studied by Principal Component Analysis (PCA) and Ascending Hierarchical Classification (HAC). The relationships between the different quantitative variables were assessed through the Pearson correlation matrix (n-1). A Discriminant Factor Analysis (DFA) was performed to best discriminate between individuals according to the classes obtained from the HAC. The confusion matrix was used to check the quality of the classification obtained.

Results:-

Isolates obtained and characterized

One (01) pure isolate of *Alternaria porri* was obtained on each of the 50 analyzed samples. The codes and provenances of these isolates are listed in Table 3. Of the 50 isolates, 10 come from the Sahelian zone, 26 from the North-Sudanian zone and 14 from the South-Sudanian zone. The village of Di presented most of the isolates (Table 2).

Table 2:- Isolates obtained from each prospected zone.

Climatic zones	Provinces	Villages/sites	Isolate code
Sahelian Zone (SZ)	Yatenga	Tougou	E001
			E005
		Toessin	E007
			E009
		Goinré	E011
			E016
		Tougzaguè	E017
			E019
	Sanmatenga	Korsimoro	E027
			E030
Total SZ	02	05	10
North-Sudanian Zone (NSZ)	Sourou	Di	E042

			E044
			E045
			E047
			E049
			E050
			E052
			E055
			E056
			E058
		Niassan	E062
			E063
		Dèbè	E064
			E066
		Gouran	E071
			E072
	Banwa	Gnanssoumadougou	E097
			E100
		Siwikotou	E103
			E106
	Kadiogo	Ouagadougou/Art3	E032
		Ouagadougou/Art4	E036
	Ganzourgou	Mogtèdo	E022
			E024
	Oubritenga	Loumbila	E037
			E041
Total NSZ	05	10	26
South-Sudanian Zone (SSZ)	Léraba	Douna	E113
			E115
		Niantonon	E110
			E111
	Houet	Soumousso	E081
			E086
		Fô	E087
			E090
		Bambé	E092
			E096
	Bougouriba	Bapla	E074
	Ioba	Benvar	E077
			E079
			E080
Total SSZ	04	07	14
TOTAL COUNTRY	11	22	50

Variability in qualitative data

Table 3 presents the results of the variability in the mycelium color, the mycelium appearance and the shape of the margins of the colonies of the 50 isolates of *A. porri* analyzed. Five colorations of the mycelium were listed at frequencies ranging from 2% for the greenish color to 38% for the purple color (Table 3). Seventy-two percent (72%) of the isolates presented a grazing mycelium against 28% which presented a cottony mycelium (Table 3). Seventy-six percent (76%) of the isolates showed regular margins and 24% irregular margins (Table 3). The different modalities of all the qualitative variables were observed in all the regions, what indicates that these morphological variables are not aligned on the agroclimatic zones.

Table 3:- Diversity of qualitative variables within populations of *A. porri*.

Variables	Modality	Headcount by modality	Fréquency by modality (%)
Mycelium color	Whitish Gray	05	10
	Purple	19	38
	Whitish Yellow	05	10
	Greenish	01	02
	Brown Gray	09	18
	Greyish Yellow	11	22
Mycelium appearance	grazing	36	72
	Cottony	14	28
Shape of margins	Regular	38	76
	Irregular	12	24

Variability in quantitative data

Descriptive statistics

Table 4 presents the extreme values of the various quantitative variables studied at the isolate level. All variables showed differences between isolates. The greatest variation between isolates was observed in the production of micro propagules with a minimum value of 1.85 CFU/ml, a maximum value of 9.3 CFU/ml and a standard deviation of 1.83, while the smallest variation was observed in radial growth at 4 DAI with a minimum value of 1.65 cm, a maximum value of 4.03 and a standard deviation of 0.46 (Table 4). The number of bands varied from 2 to 10 bands with a mean of 4.42 and a standard deviation of 1.75.

Table 4:- Extreme values and standard deviations of quantitative variables.

Variables	Minimum	Maximum	Mean	Standard deviation
4DAI (cm)	01.65	04.03	02.90	0,453
7JAI (cm)	02.45	06.65	04.67	0,628
10DAI (cm)	03.40	08.23	06.39	0,899
Micropagules count (CFU/mL)	01.85	09.30	04.62	01,831
Number of bands	02.00	10.00	04.42	01,751

Results of Principal Component Analysis (PCA).

Results of the principal component analysis performed on the quantitative data are presented in Table 5. Three main components or main axes (F1, F2 and F3) have been identified. The principal components F1 and F2 explain 76.75% of the total variations between the isolates and were therefore retained to explain the total variability within the isolates (Table 5). Principal component analysis (PCA) showed that there are significant correlations between the quantitative variables studied (Table 5).

The correlation circle shows that axis 1 with 55.34% of the total variation is positively correlated with the characters related to the radial growth of the isolates and negatively with the number of propagules (Figure 6). Axis 2 associates a positive correlation coefficient with the number of propagules but negatively with the number of circular bands (Figure 6).

Table 5:- Contribution of the different quantitative variables to the constitution of the axes.

Variables	F1	F2	F3
4DAI (cm)	0,731	0,525	-0,194
7DAI (cm)	0,919	0,232	-0,088
10DAI (cm)	0,901	0,052	0,250
Micro propagule count (CFU/mL)	-0,467	0,628	0,620
Number of bands	0,599	-0,586	0,479
Own value	2,767	1,071	0,722
Variability (%)	55,343	21,412	14,446
% cumulative	55,343	76,755	91,201

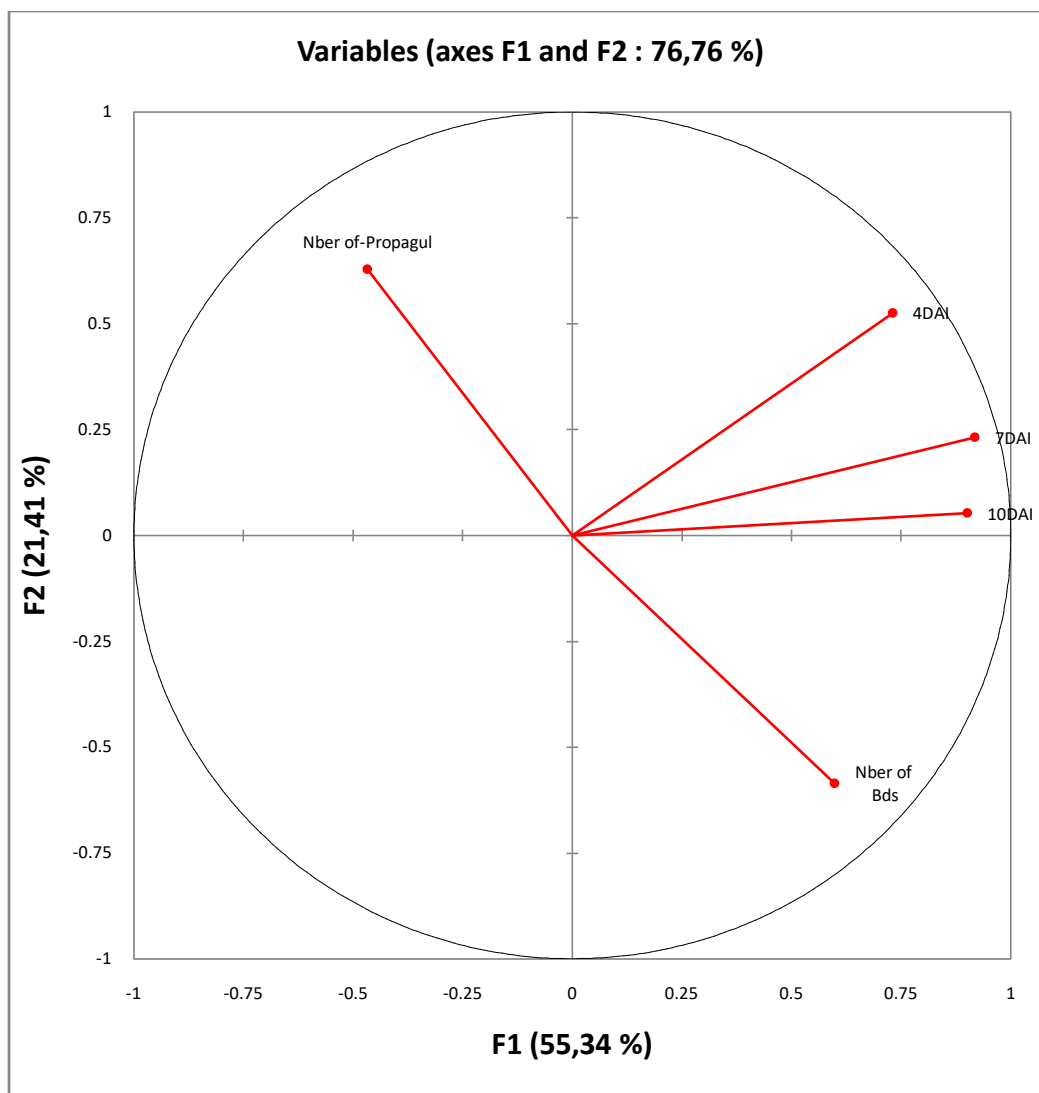


Figure 6:- PCA factorial plane correlation circle of the data.

Analysis of the correlations between variables

Table 6 presents the correlations between the different variables studied with the isolates. According to this table, the number of bands formed and the growth of the fungus are positively and strongly correlated at 10 DAI. On the other hand, the growth of the fungus is negatively correlated with the production of micro propagules. The number of bands and the production of micro propagules are negatively correlated (Table 6).

Table 6:- Matrix of total correlations between the quantitative variables studied at 5% threshold.

Variables	4 DAI (cm)	7 DAI (cm)	10 DAI (cm)	Micro propagule count	Number of bands
4 DAI (cm)	1,00				
7 DAI (cm)	0,721	1,00			
10 DAI (cm)	0,545	0,821	1,00		
Micro propagule count	-0,131	-0,328*	-0,245	1,00	
Number of bands	0,130	0,339	0,544**	-0,345*	1,00

*significant, ** verysignificant.

Hierarchical Ascending Classification (HAC)

The results of the ascending hierarchical classification shows that all the fifty (50) isolates analyzed can be grouped into three classes. The first class (class1) composed of thirty-four (34) isolates, is characterized by individuals with

low mycelial growth but a high capacity for the production of micro-propagules. This class contains isolates whose number of propagules is greater than the average for all isolates. On the other hand, the variables such as the number of circular bands and the radial growth are lower than the average. The second class (Class 2) composed of four (4) isolates, is characterized by individuals with strong mycelial growth and a low capacity for the production of micro propagules. A third class (class 3), composed of twelve isolates, is characterized by intermediate individuals in terms of growth and production of micro propagules (Table 7). These results can be summarized in the dendrogram presented in Figure 7.

Table 7:- Mean values of variables by class.

Classes	Number of isolates	4 DAI (cm)	7 DAI (cm)	10 DAI (cm)	Micro propagule count	Number of bands
1	34	2,888	4,518	6,050	25,890	3,471
2	4	3,706	6,056	7,850	14,438	5,750
3	12	2,677	4,642	6,850	18,083	6,667

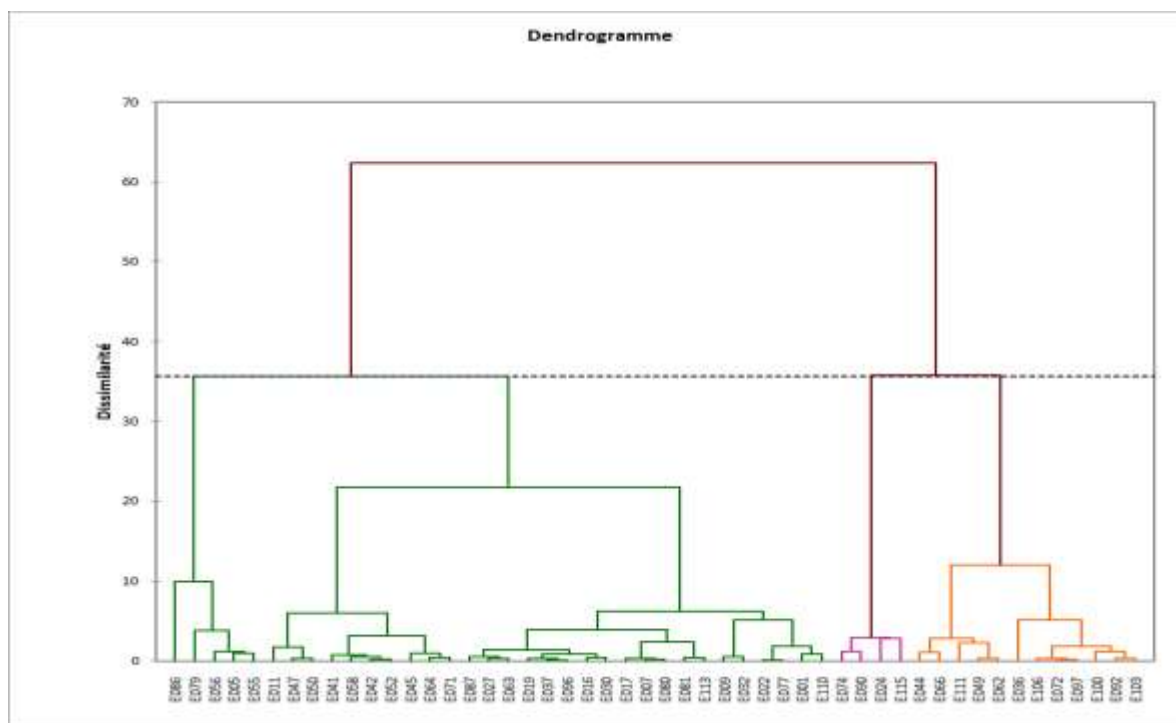


Figure 7:- Dendrogram of Hierarchical Ascending Classification of Isolates.

Comparison of classes and provenances of isolates

Table 8 presents the isolates according to their origin and their belonging to the three classes. This table shows that 26.47% of class 1 individuals are found in the South-Sudanian zone, 29.41% in the Sahelian zone and 44.11% in the North-Sudanian zone (Table 8). Class 2 individuals are mainly found in the South-Sudanian zone (75%) and the remaining 25% in the North-Sudanian region. The individuals of class 3 are mainly found in the North-Sudanian zone (83.33%) and the remaining 16.66% in the South-Sudanian zone. The isolates from the Sahelian zone all belong to class 1. Class 2 seems typical of the South-Sudanian zone and class 3 typical of the South-Sudanian zone. The first-class individuals are found throughout the country.

Table 8:- Geographical distribution of the isolates of the three classes.

Agro-climatic zones	Provinces	Villages/sites	Isolate code
Sahelian Zone (SZ)	Yatenga	Tougou	E001 (Class 1)
			E005 (Class 1)
		Toessin	E007 (Class 1)
			E009 (Class 1)

		Goinré	E011 (Class1)
			E016 (Class1)
		Tougzaguè	E017 (Class1)
			E019 (Class1)
	Sanmatenga	Korsimoro	E027 (Class1)
			E030 (Class1)
Total SZ	02	05	10
North-Sudanian Zone (NSZ)	Sourou	Di	E042 (Class1)
			E044 (Class 3)
			E045 (Class1)
			E047 (Class1)
			E049 (Class 3)
			E050 (Class1)
			E052 (Class1)
			E055 (Class1)
			E056 (Class1)
			E058 (Class1)
		Niassan	E062 (Class 3)
			E063 (Class1)
		Dèbè	E064 (Class1)
			E066 (Class 3)
		Gouran	E071 (Class1)
			E072 (Class 3)
	Banwa	Gnanssoumadougou	E097 (Class 3)
			E100 (Class 3)
		Siwikotou	E103 (Class 3)
	Kadiogo		E106 (Class 3)
		Ouagadougou/Art3	E032 (Class1)
		Ouagadougou/Art4	E036 (Class 3)
	Ganzourgou	Mogtédo	E022 (Class1)
			E024 (Class 2)
	Oubritenga	Loumbila	E037 (Class1)
			E041 (Class1)
Total NSZ	05	10	26
South-Sudanian Zone (SSZ)	Léraba	Douna	E113 (Class1)
			E115 (Class 2)
		Niantonon	E110 (Class1)
			E111 (Class 3)
	Houet	Soumouso	E081 (Class1)
			E086 (Class1)
		Fô	E087 (Class1)
			E090 (Class 2)
		Bambé	E092 (Class 3)
			E096 (Class1)
	Bougouriba	Bapla	E074 (Class 2)
	Ioba	Benvar	E077 (Class1)
			E079 (Class1)
			E080 (Class1)
Total SSZ	04	07	14
TOTAL COUNTRY	11	22	50

Homogeneity within classes

An analysis of the results of the distances per class shows that class 2 is the most homogeneous class because it has the lowest maximum distance to the barycenter (Table 9).

Table 9: -Characteristic values of distances within classes.

Classes	1	2	3
within-class variance	83,272	17,106	48,790
Minimum distance to barycenter	0,684	1,357	1,461
Mean distance to barycenter	7,480	3,177	6,191
Maximum distance to barycentre	20,638	5,431	11,581

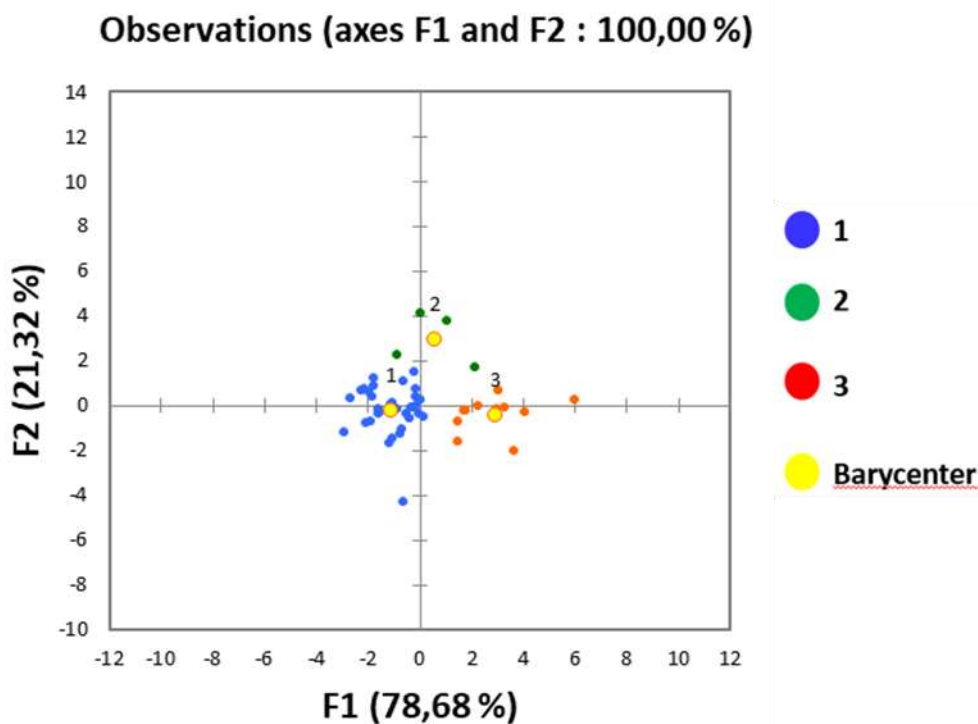
Verification of the identified classes to which isolates belong to

To find the most discriminating variables with respect to the determined classes, the discriminant factor analysis (DFA) was carried out. The three classes from the ascending hierarchical classification (HAC) were used as categorical variables in this analysis. The test of equality of class means reveals that all the tested variables make it possible to discriminate between the groups (Table 10).

Table 10: -Isolate class mean equality test.

Variable	Lambda	F	DDL1	DDL2	p-value
4 DAI (cm)	0,681	10,995	2	47	0,000
7 DAI (cm)	0,561	18,397	2	47	< 0,0001
10 DAI (cm)	0,621	14,346	2	47	< 0,0001
Micro propagule count	0,789	6,283	2	47	0,004
Number of bands	0,346	44,517	2	47	< 0,0001

The two canonical components of the AFD made it possible to bring out the classification at 100%, the first component holding 78.68% against 21.32% for the second component. Figure 4 presents the representation of the isolates in the $\frac{1}{2}$ plane of the DFA.

**Figure 9:** - Representation of the different classes of isolates in the canonical discriminating plane formed by canonical components 1 and 2.

Discussion:-

Finding effective, sustainable and ecological tools to control purple blotch disease of onion in Burkina Faso appears to be a major challenge in strengthening this agricultural sector which ensures the survival of several thousand people. To meet this challenge, it is imperative to gather some basic knowledge of the incriminated pathogen, in particular its morphological, genetic and pathogenic variability.

Analysis of qualitative data reveals that the color of the mycelium was the most prevailing variable. Indeed, six (06) colorations of *A. porri* colonies on PDA culture medium with a predominance of the purple coloration (38%) were observed. The purple color was the most common. This color, also observed on infected leaf lesions, could indicate a basic characteristic of *A. porri*. This characteristic could be a secondary metabolite which intervenes in the pathogenetic process of the fungus. These results are consistent with those of several researchers who have studied the morphological variability of this fungus in other agrosystems. Prakasam, (2010) indicated the existence of six colorations within populations of *A. porri* from India grown on the same culture medium. Mohsin et al. (2016a) also observed six colorations in populations of *A. porri* cultured on PDA medium with a predominance of purple color, in Bangladesh. Several other studies conducted on the morphological variability of *A. porri*, such as Pusz (2009), Hubballi et al., (2011), Pryor and Michailides (2012) have led to the same results. Authors like Chowdappa et al (2012), however, observed unique orange pigmentation of *A. porri* isolates in India. The variability in coloring observed could be explained by a variability in the secondary metabolites secreted by the fungus because this criterion was considered in the differentiation of pathotypes of the genus *Alternaria* (Lakshmi et al., 2014). The color of the mycelium of *A. porri* on PDA medium is therefore variable, which makes the success of a diagnosis of the pathogen on the basis of the color of its mycelium on this medium uncertain. The appearance of the mycelium was grazing or cottony and the shape of the margins was regular or irregular within the analyzed 50 isolates. These results were also observed by Rai and Kumari (2009), Prakasam (2010), Mohsin et al. (2016a) and Chowdappa et al. (2012). The variability of all these qualitative data may indicate genetic variability within isolates (Chowdappa et al., 2012) and hence, variability in the pathogenesis of the fungus. However, we must keep in mind that phenotypic characters are most often influenced by environmental conditions (pH, temperature, humidity) and may be responsible for the observed variability.

It should be noted that this variability observed at the level of the color, shape and appearance of the colonies of *A. porri* cultured on PDA medium is not aligned with the geographical area of origin of the isolates. This result was also reported by Prakasam et al. (2010) and Mohsin et al. (2016a) respectively on the populations of India and Bangladesh. In general, the genus *Alternaria* presents a great morphological variability which leads some researchers to attempt to reorganize the taxon into subgenera (Mohsin et al., 2016b). Likewise, Goyal et al., (2011) observed extensive variation in morphological variables of the isolates of *A. brassicae*. Sofi et al. (2013) found a strong variability within populations of *Alternaria mali* associated with leaf blotch of apple.

The fifty isolates of *A. porri* from Burkina Faso showed variation in their growth, the production of micropropagules and the number of growth bands on the PDA medium. The radial growth at the 10th DAI varied from 3.4 cm to 8.22 cm with a standard deviation of 0.89. The production of micro propagules varied from 1.85 to 9.3 CFU/mL. Class 1 isolate E050 recorded the highest production of micro propagules. The number of bands varied from 2 to 10 bands with a mean of 4.42 and a standard deviation of 1.75. All these results corroborate with results obtained in other localities. Priya et al. (2018) found an average radial growth of between 4.5 and 8.8 cm after 10 days of culture and indicated that the PDA culture medium was suitable for the cultivation of *A. porri*. The mycelia characteristics of *A. porri* were found to be variable from smooth to fluffy and whitish to dark olivaceous (Shahnaz et al., (2013). Also, isolates of *A. porri* showed significant variation in colony colour, growth pattern, margins shape (Chethana et al., 2018). Principal component analysis (PCA) showed that there are significant correlations between the quantitative variables studied. This implies significant variation existing between isolates. A negative correlation was observed between the number of micro propagules and the number of circular bands. However, a positive correlation exists between the number of circular bands and radial growth. These results indicate that the rapid growth of an isolate does not necessarily imply its virulence. This could be explained by the presence of non-viable mycelium during the growth of the fungus. The results of the Ascending Hierarchical Classification (AHC), indicate that the variables affecting virulence (Number of propagules) and the variables related to growth capacity (radial growth) are those which discriminate the classes. Thus, our results show a class of isolate with rapid growth (7.85cm) (class 2), a class with intermediate growth (6.85cm) (class 3) and a class with low growth (6.05) (class 1). Our results are in agreement with those of Chowdappa et al. (2012) who found three classes of isolates (fast, intermediate and slow) when analyzing the diversity of *Alternaria porri*. Class 1 includes 34 isolates. Isolates of this class have a higher

number of propagules than other classes. However, isolates of this class have slow radial growth and fewer circular bands than other groups. Class 2 with 4 isolates includes individuals with rapid growth and a lower number of propagules than the other groups. Several researchers have reported morphological and pathogenetic variability among isolates of *Alternaria* spp. (Singh et al., 2003; Tetarwal et al., 2008). It would appear that the variation among isolates is not a function of geographic location since isolates were collected from different remote sites and each class includes isolates from multiple collection sites. However, our results indicated that the individuals of class 1 are found in all areas while those of class 2 seem specific to the South Sudanian region and those of class 3 to the North Sudanian region. Phenotypic traits are influenced by environmental conditions, so they may be responsible for such diversity (Sofi et al. 2013).

Conclusion:-

Today in Burkina Faso, onion is the most produced vegetable speculation. The study which we carried out aimed to characterize isolates of *Alternaria porri* responsible for purple blotch of onion. *Alternaria porri* isolates have been studied to understand their morphological characteristics. The results of the work revealed that, based on morphological variables, high morphological variability was observed in *Alternaria porri* isolates collected in Burkina Faso. Hierarchical ascending classification presented isolates into three classes based on radial growth. The discriminant factor analysis confirmed that in the constituted classes, all the variables made it possible to discriminate the three classes. In view of the results of this study, it would be wise to study diversity at the molecular level. This could help us reveal the true nature of this fungus from Burkina Faso and leading to the implementation of appropriate and sustainable management of onion purple blotch disease. It would also be wise to evaluate the biochemical activity of the fungus in order to know the impact of mycotoxins on animal and human health.

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