International Journal of Agriculture Innovations and Research
Volume 4, Issue 4, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received : 11/01/2016 | Accepted on : 25/01/2016 | Published : 04/02/2016

Evaluation of Resistance of Seven Pepper Varieties to Sclerotinia Caused by *Sclerotium Rolfsii*, in the Locality of Songon -Te, in the South of Cote d'Ivoire

Bolou Bi Bolou Antoine[1], Abo Kouabenan[2] Kouamé Koffi Gaston[1], Kone Daouda[1]
1 Laboratory of Plant Physiology, Faculty of Biosciences, Felix Houphouet Boigny University,
22 BP 582 Abidjan 22, Côte d'Ivoire
2 Laboratory of Phytopathology and Plant Biology, Department of Training and Research Agriculture and Animal Resources (DTR-AAR), National Polytechnic Institute of Felix Houphouet-Boigny (INP-HB), BP 1313 Yamoussoukro, Côte d'Ivoire

Abstract **– A study in semi-controlled conditions and in the open field, has been conducted on seven pepper varieties to test their resistance against sclerotinia caused by *Sclerotium rolfsii* at Songon- Té, in southern Côte d'Ivoire. Pepper varieties tested are: Tiela, Super, Nickly, Batur, Tongla, Demon and Yolo Wonder. At the end of the study, the witness Yolo Wonder, known for its sensitivity, has been very vulnerable to disease, with a severity rate of 90%; Batur and Tongla were likely, with respective severity rate of 61.13 ± 0.19% and 52.70 ± 0.14%, while three varieties that are Tiela, Super and Demon have been moderately likely to the attack of the pathogen agent, with respective severity rates of 42.51 ± 0.91%; 25.96 ± 0.38% and 21.68 ± 0.84%. As for the variety Nickly, it has been stronger against sclerotinia, with a severity rate of 0%.**

Keywords **–** ***Sclerotium rolfsii*****, Pathogenicity, Pepper, Resistance, Open field.**

I. INTRODUCTION

Food plays a very important role in nutrition and human health and the maintenance of the body. Among these foods, the pepper (*Capsicum* spp) is very important for small farmers in developing countries. The Pepper, tangy, today dominates all world trade of spices and the sweet pepper has become a popular crop in the tropics [1]-[2]. More than 25 species of pepper are described of which only five (*C. annum* L., *C. Chinense* Jacq., *C. frutescens* L., *C. baccatum*.and *C. Pubescens* Keep.) are domesticated or cultivated [3]-[4]. According to FAO statistics, in 2013, the pepper is grown on almost 3.8 million hectares of which 3.3 million are in developing countries. It is more cultivated in Asian countries, with 2.5 million hectares and 0.8 million hectares for those of Africa. The farming of pepper is practiced the whole year and everywhere in Côte d'Ivoire but requires some time the watering against season [5]. However, this culture is subject to significant pest pressure. At least 10% of world production is still lost because of diseases, which leads to a considerable reduction in producers' incomes [6]. The farming of pepper suffers from anthracnose or fruits rot, wilting, falling leaves and powder mildew. Among these diseases, annulus rot caused by *Sclerotium rolfsii* is of economic importance and more restricting for the farming of pepper [7]. The damage is particularly charged that the severity of the disease is high in the fields. The lost of production may rise between 10- 25% or even above 81% [8]-[9]. It is very difficult to control telluric fungus, especially *S. rolfsii* through conventional methods such as applying fungicides, farming methods etc. First, the application of chemical fungicides is expensive, harmful and hazardous for the environment and human health [10]. The World Health Organization (WHO) and the UN Environment Program assess that each year three million workers in agriculture in developing countries, are severely poisoned by pesticides of whom 18,000 die [11]. The development of appropriate technologies resulting from the appropriate use of natural resources is strongly desired in agriculture. One of these techniques is the use of disease-resistant varieties which is the main purpose of this study.

II. MATERIAL

A. *Plant Material*

The plant material is made up of seven pepper varieties whose characteristics are reported in Table I.

Table I: Characteristics and origins of different varieties of pepper tested

Variety	Characteristics of fruits	Origin
Yolo wonder	Fruit hypogeous wide gark green colour	France (INRA, Avignon)
Tiela	Small fruit epigeous dark green colour	East-West Seed International (Netherland)
Tongla	Small fruit epigeous dark green colour	
Super	Long fruit epigeous dark green colour	
Batur	long fruit epigeous olive green colour	
Nickly	Medium fruit hypogeous green colour	
Demon	Small fruit epigeous dark green colour	

B. *Fungal used*

The pathogenic fungi used in this study is *Sclerotium rolfsii*. It has been isolated from an infected pepper plant on an experimental plot in Songon. This fungi produces mycelium whitish visible to collar plants.

International Journal of Agriculture Innovations and Research
Volume 4, Issue 4, ISSN (Online) 2319-1473

III. METHODS

A. *Verification Test of contamination of the study site*

A preliminary study has been conducted on the test plot to verify the presence of pathogens. The test site belongs to the area called agro-ecological zone 1 (AEZ I), gathering the humid forest regions of Ivory Coast. This agro-ecological zone has an average temperature of 29 ° C, with a very high rainfall of 1400 mm to 2500 mm (Table II). There, an experiment has been conducted using 20 pepper plants aged 45 days of the variety Yolo wonder that have been planted randomly. After 30 days, the plants showed the first symptoms of sclerotinia (appearance of mycelium collar). Diseased plants have been collected for isolation of pathogens.

Table II: features of seven agro-ecological zones (AEZ) in Côte d'Ivoire [12]

AEZ	Characteristics	Altitude (m)	Pluviometry (mm)	Average annual temperature in °C (Standard deviation)	Experimental site
I	Southern dense rainforest area	0-200	1400 - 2500 (bimodal)	29 (5.6)	Songon Té
II	Western dense rainforest area	~1000 (Daloa)	1300- 1750	23.5 (13.4)	
III	Western semi-montainous forest area	>1000 (Man)	1300- 2300	24.5 (7.7)	
IV	Semi deciduous dense rainforest area	0-200	1300-1750	23.5 (13.4)	
V	Transitional forest area	300-600	1300-1750 (unimodal)	23.5 (13.4)	
VI	Humid tropical savanna area	300- 500	1150-1350 (unimodal)	26.7 (1.1)	
VII	Dry tropical savanna area	300-500	1150-1350	26.7 (1.1)	

B. *Isolation and identification of fungus*

Samples of diseased plants were put in sterile bags. They have been sent to the laboratory for the isolation of pathogenic fungi responsible for necrosis. The samples were rinsed with tap water and cut into small pieces (explants) and sterilized with sodium hypochlorite to 10%. Then, the explants have been rinsed with sterile distilled water and towel-dried using sterile filter paper. Five explants have been placed in a Petri dish containing PDA medium and incubated at 28 ° C. Fungal colonies produced around the explant have been harvested for transplanting into new Petri dishes. The end of the mycelium has been takenand transferred to a new culture medium in sterile Petri dishes until a single coloringthallus fungi. Thallus fragments have been collected to be observed using a light microscope brand AMSCOPE equipped with a camera.

C. *Pathogenicity test*

To check the pathogenesis of *Sclerotium rolfsii* under cover testing (semi-controlled conditions) have been achieved. In fact, 25 sclerotia have been inoculated all around each plant, with five sclerotia per hole. The five holes performed around plants have a depth of 2 cm each. The plants have been watered throughout handling. All varieties have been used to conduct the test of pathogenicity and Yolo Wonder has been taken as a witness because of its high sensitivity to disease. The handling has been performed three times with 20 plants of each variety.

D. *Implementation of open field resistance tests*

Nurseries of different varieties have been made in plates seedlings of 72 cells. After 45 days in the nursery, seedlings have been planted on ridges ten meters long and one meter wide. Each ridge of earth has 20 plants of each variety. In total 420 plants at the rate of 60 plants of each variety have been planted. The plants were far away from each other of 0.50 m with line spacing of one meter. The observations were performed every week for three months. The tests have been repeated three times. The interruption of observations has been sanctioned by calculating the disease severity rate. The symptom assessment has been performed basing on a rating scale of symptoms proposed by the formula of Townsend-Heuberger [13]. This rating scale comprises four values from 0 to 3:

0: Healthy Plant
1: Yellowing plants
2: Wilting plants
3: Death or total defoliation of plants

$TS\ (\%) = 100\ [\ \sum(nv)/\ (NV)]$

n: degree of infection according to the scale [14]
v: Number of plants per category
N: Total number of tested plants
V: The highest degree of infection.

The tests have been performed three times with 20 plants of each variety placed on two rows of 10 plants. The data collected during the different tests have been subjected to analysis of variance using Statistica 7.1 software. 1 and 2 post ANOVA analyzes have been performed for the classification of different averages. The test of Newman - Keuls has been used at the 5% threshold.

International Journal of Agriculture Innovations and Research
Volume 4, Issue 4, ISSN (Online) 2319-1473

IV. Results

A. Pathogenicity of Sclerotium rolfsii behavior of different pepper's varieties in semi controlled condition

After the inoculation of different varieties, the witness Yolo Wonder has been quickly attacked from the 15th day. The other varieties began to show the first symptoms of the disease from the 30th day after inoculation. The severity rates were calculated 60 days after inoculation of varieties. The values of the severity rates have allowed us to classify the different varieties into four categories. Yolo Wonder has been very sensitive with a 100% severity rate. As for the varieties Batur and Tongla, they were likely, with severity rate of the disease of 61.13 ± 0.19 % and 52.70 ± 0.14 % respectively. Less than the first two varieties, three other varieties showed low severity rates which are 42.51 ± 0.91 % for Tiela 25.96 ± 0.38 % for Super and 21.68 ± 0.84% for Demon. Conversely, the Nickly variety did not present symptoms of sclerotinia. Its severity rate was zero (Figure 1).

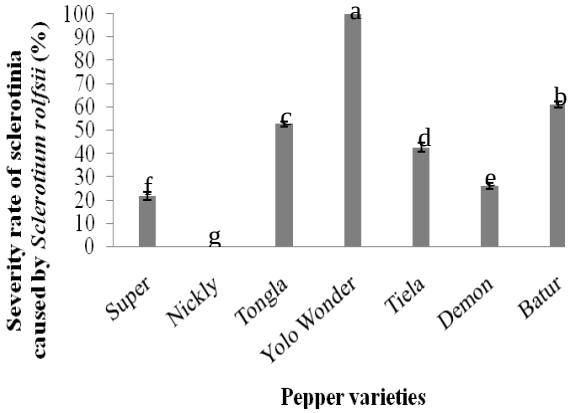

Fig. 1. Behavior of pepper varieties under controlled conditions, facing sclerotinia

B. Behavior of different pepper's varieties in the open field

Seeing that the evaluation in the open field may involve several parameters, it has been judicious to wait for the manifestation of different characteristic symptoms before making a good assessment. Thus, the variety Nickly responded well to the attacks of *S. rolfsii*, as in the tests in shelters. The attack rate of this variety is 1.58 ± 0.46 %. The witness, Yolo Wonder, with a severity rate of 90 ± 1.61%, has always been the most sensitive of varieties. The reactions of other varieties, facing the attack of *S. rolfsii* have been the same as in the *in vivo* studies (Figure 2). By and large, it was found an increase of 12% of the *in vivo* disease severity rates for Super, Demon, Tiela and Tongla in comparison to rural manifestations which are respectively 34 ± 1.6 %, 38.91 ± 1.46 %, 55.3 ± 1.44 and 64.8 ± 1.28 %. Only the severity rate of the variety Batur has increased by 16 %.

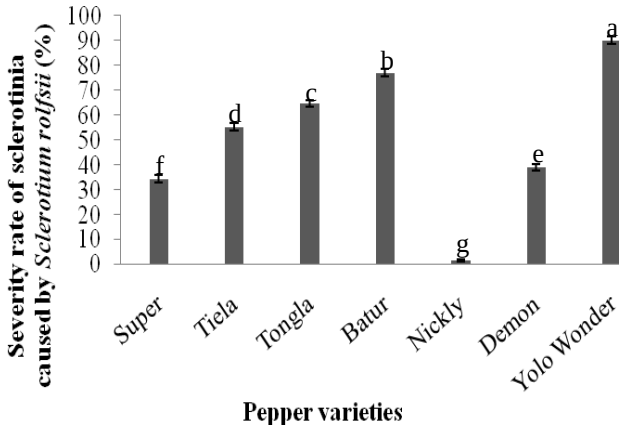

Fig. 2. Severity rate of sclerotiniaonthe different varieties of pepper

V. Discussion

The experiments lead in this study, took place in the locality of SongonTé , situated in the so-called humid forest zone ZAE I. This area is known for its very high humidity, with a rainfall of 1400 to 2500 mm per year and an average temperature of 29 ° C ± 5.6 [12]. However, ecological conditions for a good mycelial growth of *Sclerotiumrolfsii* are: a high temperature (25 -35 ° C), low or acidic pH (3-6) and high humidity [15]. The environmental conditions of this area would be the cause of the increase in the severity rate of the disease at least 12% in the open field compared to controlled conditions for different varieties tested. Thus, the increase of this rate of severity of 12% of the disease, for varieties of Super, Demon, Tongla and more than 16 % to Batur is due to environmental conditions of the experimental site that are favorable to the Proliferation of *S. rolfsii*. These results agree with those of [16], who indicated that weather conditions play a very important role in infections and rainy seasons are favorable for a better growth of *S. rolfsii* in peanut farming. They also assert that the combination of these two factors is much favorable to the manifestation of the disease in the open field. The severity rate of the disease is between 1.58% and 90%, with an average of 51.66 %. Based on the work by the same authors, any variety obtaining a severity rate less than 20 % is said to be resistant. So, the variety Nickly would be the only resistant with a severity rate of 1.58% obtained in our study.

The resistance of plants against attacks from pathogenic agents depends on a plurality of factors. Many authors have demonstrated that there is a distinct correlation between the degree of resistance and the presence of phenols in the tissues of a plant [17]-[18]. Thus, the enzymes phenylalanine ammonia lyase and peroxidase are oxidative enzymes involved in the biosynthetic process of phenol associated with the infections of pathogens. The accumulation of enzymes such as phenylalanine ammonia lyase, peroxidase and polyphenol oxidase in plant tissues after their inoculation by *S. rolfsii* prevented the

infestation of these ones. This resistance caused by the presence of these enzymes in the tissues of inoculated plants has been demonstrated by [19] on tea plants. Thus, the resistance of the variety Nicklywould be due to the accumulation of phenylalanine ammonia lyase, peroxidase and polyphenol oxidase in its tissues. [20] have demonstrated that oxidative enzymes such as polyphenoloxidase and peroxidase are involved in the resistance of plants against pathogens. The total resistance of the variety Nickly and partial of the varieties Super,Demon and Tonglawould be due by the presence of oxidative enzymes in their tissues. *S.rolfsii* is a fungi which secretes oxalic acid in the host plant tissue during the infection process. However, oxalic acid is a toxin that binds calcium to prevent calcium association with pectic compounds in the guard cells. This link would cause a decrease in the pH of guard cells and as a result, would facilitate the activity of cellulases and endopolygalacturonases [21]. The Secretion of endopolygalacturonases would destroy polysaccharides for inhibiting the action of polygalacturonases. Plants produce a defense mechanism by which the action of pathogenic is specifically inhibited by a group of guard cells called inhibitory proteins polygacturonases [22]. These proteins have already been characterized in several plant species [23]. The resistance of different pepper varieties against *S. rolfsii*, which is one of the oxalic acid producing fungus would be due to the presence of inhibitory proteins of polygalacturonases.

VI. Conclusion

At the end of this study, conducted in semi-controlled conditions and open field, it brings out that a variety of pepper, and especially the Nickly resists or tolerates sclerotinia caused by *S. rolfsii*. In fact, the results under controlled conditions and in open fields are well correlated for this variety which expressed severity rate of attack from 0 to 1.58 %. Other varieties, whose severity rates are variable in both studies conditions, have values beyond 20 %. They can therefore be considered as resistant. It therefore brings out in this study, that only the use of Nickly variety can be considered in an integrated struggle strategy against sclerotinia. Considering the interest in the use of resistants varieties, you must continue screening a larger number of pepper varieties, integrating the elucidation of biochemical and genetic mechanisms involved in resistance, if proved.

References

[1] Dias G, Gomes V, Moraes T, Zottich U, Rabelo G, Carvalho A, Moulin M, Gonçalves L, Rodrigues R, Da Cunha M, (2013). Characterization of *Capsicum* species using anatomical and molecular data. Genetics and Molecular Research. 4,1-14

[2] Y Wahyuni, Ballester A, Sudarmonowati E, Bino R, Bovy A, (2013). Secondary Metabolites of Capsicum Species and Their Importance in the Human Diet. Journal of National Products. 76, 783-793.

[3] P. Bosland and E. Votava (2000). Peppers: vegetable and spice capsicums. CABI. Publishing, Wallingford, United Kingdom, first edition Crop production sciences in horticulture 204 p.

[4] L. Costa, R. Lopes, M. Lopes and A. Figueiredo (2009). Cross compatibility of domesticated hot pepper and cultivated sweet pepper. Crop Breeding. Applying and. Biotechnology. 9, 37-44

[5] L. Fondio, C. Kouamé, A. H. Djidji and S. Aïdara (2009). Bien cultiver le piment en Côte d'Ivoire. Fiche technique du Centre National de Recherche Agronomique (CNRA) 4 p. info.sqr@cnra.ci

[6] R. Strange and P. Scott. (2005). Plant Disease: A Threat to Global Food Security.Annual. Review of Phytopathology 43:83-116. r.strange@ucl.ac.uk

[7] Kalmesh M. and Gurjar R. (2001). *Sclerotium rolfsii* a new threat to chilli in Rajasthan. Journal of Mycology and Plant Pathology, 31: 261.

[8] V. Mehan, C. Mayee. D. McDonald, N. Ramakrishna and S. Jayanthi (1995) Resistance in groundnut to *Sclerotium rolfsii* caused stem and pod rots. International journal of pest Management, 41: 79-82.

[9] A. Evueh, G. Ekuoyah, O. Osemwegie, H. Idress and O. Ogbebor, (2011). Evaluation of paphylloplane fungi biocontrol agent of Corynespora leaf fall disease of rubber (Hevea brasiliensis Muell. Arg.). World Journal of Fungal and plant Biology 2, 1-5.

[10] Z. Punja (1985). The biology, ecology, and control of *Sclerotium rolfsii**Error! Bookmark not defined.*. Annual Review of Phytopathology 23: 97 - 127.

[11] G. Miller, (2004). Sustaining the Earth, 6th edition. Thompson Learning, Inc. Pacific Grove California, Chapter 9, pp. 211- 216.

[12] B. Halle, V. Bruzon, (2006). Profil Environnemental de la Côte d'Ivoire. Rapport final. Consortium AFC, AGRIFOR Consult: 133p.

[13] A. Erkilic, B. Guven and D. and Akgul, (2006). Effects of some plant activators and plant materials on stem rot disease of peanut and pepper caused by *Sclerotium rolfsii*. Journal of Turkish Phytopathology, 35, 15-18.

[14] C. Le, R. Mendes, M. Kruijt and J. Raaijmakers (2012). Genetic and phenotypic diversity of *Sclerotium rolfsii* in groundnut fields in central Vietnam. *Plant Disease*, 96, 389-397.

[15] J. Sinclair (1982). Compendium of soilbean diseases. American. phytopathology. Society. St. Paul, 104 p.

[16] S. Bera, S . Kasundra, J. Kamdar, B. Ajay, L. Chuni , L. Thirumalasmy , P. Dash, A. Maurya. (2014). Variable response of interspecific breeding lines of groundnut to *Sclerotium rolfsii* infection under field and laboratory conditions. Electronic Journal of Plant Breeding, 5, 22-29

[17] R. Sridhar and S. Ou (1974). Phenolic compounds detected in rice blast disease.Biology of. Plant 16, 67-70.

[18] R. Hammerschmidt and R. Nicholson. (1977). Resistance of maize to anthracnose: Change in host phenols and pigments. Journal of Phytopathology 67, 251-258.

[19] Indramani B, Bishwanath C, (2010). Defense response triggered by *Sclerotium Rolfsii* in tea plants. ECOPRINT 17, 69-96.

[20] C. Chen, R. Be`langer, N. Benhamou and T. Pautitz, (2000). Defense enzyme induced in cucumber roots by treatment with plant growth promoting rhizobacteria (PGPR) and *Pythium aphanidermatum.* Journal of Physiology, Molecular and Plant Pathology. 56, 13-23.

[21] A. Ghaffar (1976). Inhibition of fungi as affected by oxalic acid production by *Sclerotium delphinii*. Pakistan Journal of Botany. 8, 69-73.

[22] F. Cervone, M. G. Hahn, G. De Lorenzo, A. Darvill and P. Albersheim, (1989). Host-pathogen interactions XXXIII. A plant protein converts a fungal pathogenesis factor into an elicitor of plant defence responses. Plant Physiology 90, 542–548.

[23] S. Shivashankar, C. Thimmareddy, and K. Tapas. (2010), August). Polygalacturonase inhibitor protein from fruits of anthracnose resistant and susceptible varieties of Chilli (*Capsicum annuum* L) Indian Journal of Biochemistry & Biophysics 47, 243-248

AUTHORS' PROFILES

First A. Author BOLOU BI Bolou Antoine
An Ivorian national born on 25th December 1981 in Zuénoula (Côte d'Ivoire).His educational background issummarized as: Master degree of Biodiversity, climate change and conservation of ecosystems at Nangui Abrogoua University in option of Plant Pathology. Since 2012, he enrolled in Plant Physiology courses at Felix Houphouet Boigny University to earn a PhD in Plant Pathology. Email: bolantoine@yahoo.fr

Dr ABO Kouabenan.
Assistant professor in the Laboratory of Phytopathology and Plant Biology, Department of Training and Research Agriculture and Animal Resources (DTR-AAR), National Polytechnic Institute of Felix Houphouet-Boigny (INP-HB), BP 1313 Yamoussoukro, Côte d'Ivoire
Email: abok65@yahoo.fr

Ph.D. KOUAME Koffi Gaston
Assistant Professor of Plant pathology at Félix Houphouët Boigny University,22 BP 582 Abidjan 22, Côte-d'Ivoire.
Email: k.koffigaston1@yahoo.fr

Prof. KONE Daouda
Professor of Plant pathology at Félix Houphouët Boigny University, 22 BP 582 Abidjan 22, Côte-d'Ivoire.
Email: daoudakone2013@gmail.com