

Research Article

Response of Nine Cultivars of Pepper (*Capsicum* spp.) to Infection by Four Viruses under Natural Field Conditions in the Coastal Savanna Zone of Ghana

¹A.S. Appiah, ²E.K. Quartey, ²H.M. Amoatey, ²W. Nunekpeku, ²M. Owusu-Ansah and ³S. Ofori,

¹Biotechnology Center,

²Nuclear Agriculture Center,

³Radiation Entomology and Pest Management Center, Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, P.O. Box LG 80, Legon, Accra, Ghana

Abstract: A study was conducted to evaluate the reaction of two local and seven exotic pepper cultivars to four viral diseases in the coastal savanna zone of Ghana. The objective was to evaluate the response of the pepper cultivars to the viruses under natural field conditions. The plants were assessed for symptom severity as well as disease incidence. Symptom expression varied among the cultivars, with severity ranging from 0.5% at 4 Weeks after Transplanting (WAT) to 47.2% at 14 WAT. Disease incidence also varied significantly among the cultivars and ranged from 1.1% at 4 WAT to 61.3% at 14 WAT. Enzyme-Linked Immune Sorbent Assay (ELISA) was performed against PVMV, TMV, CMV and PMMV for each cultivar. The test showed the presence of all four viruses in the leaf extracts, with each cultivar infected by at least one of the four viruses. PVMV and CMV were the predominant viruses identified. Mixed infections of two or more viruses were observed in all the cultivars. TMV was detected in all cultivars except the two hybrids, Sunny F₁ and Forever F₁, while PMMV was detected in only cv. Legon 18 which had a mixed infection of all four viruses.

Keywords: *Capsicum* spp., cultivar, disease incidence, enzyme-linked immunosorbent assay, mixed infection, symptom severity, viruses

INTRODUCTION

Pepper belongs to the genus *Capsicum* and family Solanaceae (Ochoa-Alejo and Ramírez-Malagón, 2001). It is an indispensable ingredient for food preparation throughout the world (Green and Kim, 1991) and is also utilized in the pharmaceutical, cosmetic and poultry industries (Jin *et al.*, 2009).

In Ghana, pepper is widely produced for both local consumption and export to the European market. Currently, Ghana is the fifth largest exporter of peppers to the European Union, where the demand has been growing by about 17% annually since 2000 (Millennium Development Authority, 2010). However, the incidence of viral diseases has become a major constraint to production.

Some 35 viruses have been reported to infect peppers worldwide causing considerable economic losses in terms of reduced fruit quality and marketable yield (Jin *et al.*, 2009). In Africa, 11 viruses have been found to infect pepper crops (Gafalla, 2003). The most important of them include Pepper Veinal Mottle Virus (PVMV) and Cucumber Mosaic Virus (CMV). Planting of resistant cultivars offers the best control measure as

the use of chemical or cultural means has not been very effective. A number of sources of virus resistance have been reported in wild and cultivated lines of pepper and several virus-resistant lines have already been released. However, some viruses, particularly Cucumber Mosaic Virus (CMV) and the leaf curl virus complex, continue to pose a challenge to breeding programmes (Green and Kim, 1994).

In Ghana, seeds of some exotic pepper varieties under various trade names are being sold to farmers by commercial seed companies for planting. Adaptability of these cultivars to the local environment especially to viruses is not yet known. There is, therefore, the need to assess the response of these cultivars to viruses present locally. This will allow the identification of resistant or tolerant cultivars for recommendation to farmers in the short term and in the long term utilized in breeding programmes to improve well-adapted local cultivars. Furthermore, it will allow for the development and evaluation of better control measures, thereby, reducing production losses and increasing the quality of the crop. This project, therefore, sought to evaluate the response of seven exotic as well as two local cultivars of pepper to four pepper viruses in the Coastal Savanna agro-ecological zone under natural field conditions.

Corresponding Author: Andrew Sarkodie Appiah, Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, P.O. Box: LG 80, Legon, Accra, Ghana, Tel.: +233-24-4164426

This work is licensed under a Creative Commons Attribution 4.0 International License (URL: <http://creativecommons.org/licenses/by/4.0/>).

MATERIALS AND METHODS

Source of seeds: Seeds of the exotic varieties used for the study namely, California Wonder (CW), Big Sun (BS), Archard (AR), Bombadier (BO), Forever F1 (FF), Sunny F1 (SF) and Antillas (AT) were obtained from Technisem, Accra. The local cultivars; Legon 18 (LE) and Anloga (AN) which were used as checks were obtained from Agrimat, Accra and a farmer's field respectively.

Nursery establishment and field planting: One hundred and fifty seeds each of the nine cultivars were sown and raised in plastic trays in a sterilized soil mixture composed of sandy loam and decomposed coconut fibre in a ratio of 2:1. The trays were kept in insect proof cages. At four weeks after emergence, the seedlings were transplanted in the field in a Randomized Complete Block Design (RCBD) with three replications. Thirty seedlings per cultivar were planted per plot at a spacing of 0.75×0.65 cm. NPK fertilizer (19-19-19) was applied as a starter solution at transplanting. Eighty grams (80 g) of fertilizer material was dissolved in 4.5 L of water and applied at 15 mL per seedling. No insecticide was used throughout the study.

Host response in the field: Phenotypic data on host reaction were recorded in terms of symptom expression following a six-point scoring scale modified after Piper *et al.* (1996). Using the symptom severity scores, percentage severity was estimated as:

$$\text{Severity, } S (\%) = \frac{\text{Number of plants } \times \text{ each degree}}{\text{Number of plants evaluated } \times \text{ highest degree}} \times 100$$

Disease incidence, which measures the extent of propagation of a disease within a given field (Agrios, 2005), was also estimated using the formula:

$$\text{Incidence, } I (\%) = \frac{\text{Number of plants (or parts)}}{\text{Total number of plants (or parts) observed}} \times 100$$

According to González-Pérez *et al.* (2011).

Virus detection: The technique of direct Double-Antibody Sandwich ELISA (DAS-ELISA) was performed following the method of Clark and Adams (1977) for the detection of Cucumber Mosaic Virus (CMV), Pepper Veinal Mottle Virus (PVMV), Tobacco Mosaic Virus (TMV) and Pepper Mild Mottle Virus (PMMV). The ELISA kits used for the virus detection were obtained from DSMZ Plant Virus Collection, Braunschweig, Germany.

Leaf extract was prepared by grinding 0.5 g of young leaves in 5 mL of General ELISA extraction

buffer containing 20 g of Poly Vinyl Pyrrolidone (PVP), 2 g of ovalbumin, 1.3 g of sodium sulphite (anhydrous), 0.2 g of sodium azide, 0.5 mL of tween 20, 8 g of sodium chloride and 0.2 g of potassium dihydrogen orthophosphate, 1.15 g of disodium hydrogen orthophosphate and 0.2 g of potassium chloride. Positive and negative controls were included on each ELISA microtiter plate for validation of the test results. Absorbance values were measured at 405 nm using spectrophotometer (Multiskan Ascent VI.25-Version 1.3.1) and a reaction was considered positive when the absorbance value was at least two times higher than that for the healthy control.

Statistical analysis: Disease incidence was obtained by making counts of infected plants for each cultivar and expressing it as a percentage of the total number of plants. The disease incidence values were arcsine transformed before Analysis of Variance (ANOVA). Similarly, percentage symptom severity values were also transformed by Arcsine before ANOVA. Data were analyzed using GENSTAT statistical software and the treatment means were separated using Tukey's pair wise comparison.

RESULTS AND DISCUSSION

The nine pepper cultivars exhibited various degrees of symptoms of viral infection. These included mosaic, yellowing, vein clearing, puckering, leaf curling, leaf distortion and stunting (Fig. 1). The symptoms were



Fig. 1: Pepper cultivars showing viral disease symptoms
 A: Anloga-mosaic; B: Antillas-leaf distortion and reduction; C: Big sun-leaf distortion; D: Archard-leaf deformation; E: Bombadier-leaf distortion and blistering; F: California wonder-mosaic, leaf reduction and distortion; G: Forever F1-mosaic, leaf rolling and reduction; H: Legon 18-leaf yellowing, vein banding and stunting; I: Sunny F1-upward cupping, mosaic and reduction in size

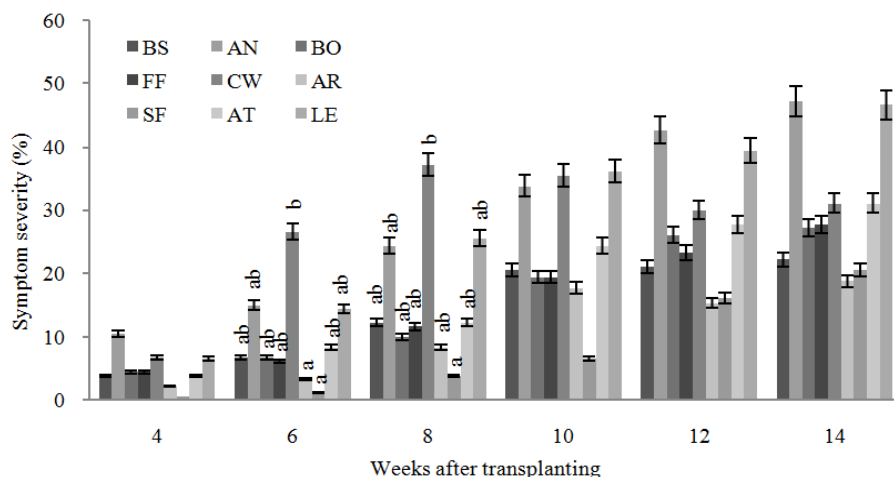


Fig. 2: Symptom severity among nine local and exotic cultivars of pepper infected by four viruses ($p \leq 0.05$)

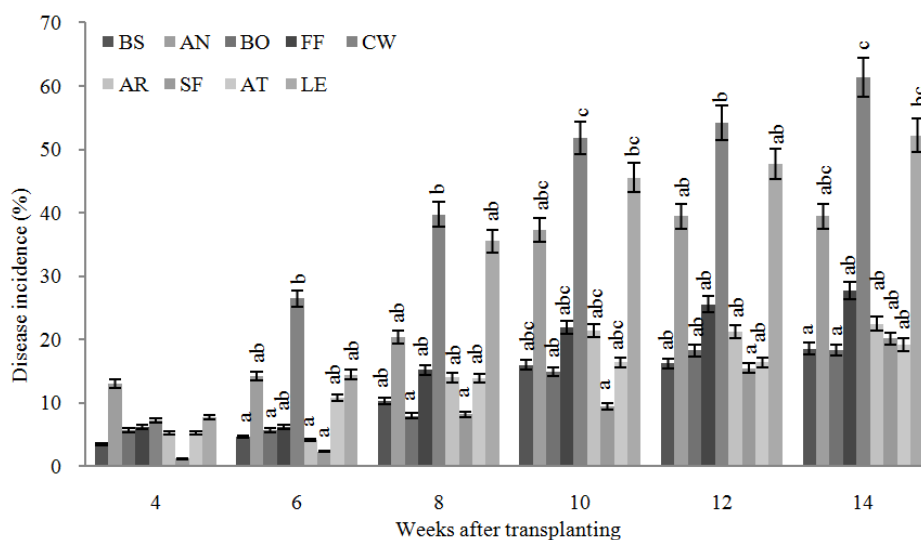


Fig. 3: Incidence of viral diseases among local and exotic cultivars of pepper

similar to those reported by Atiri (1992) and Rashid *et al.* (2007) on pepper infected by PVMV and CMV. However, symptoms alone are insufficient in virus identification since their expression may be influenced by the host cultivar, strain of virus, age of the host, environmental conditions as well as possible co-infection with other viruses (Sherwood *et al.*, 1986). Furthermore, different viruses may induce similar symptoms, while insect damage, particularly by thrips and mites, may also produce symptoms similar to virus infection (Green and Kim, 1994).

Generally, symptom expression among the different cultivars increased in severity with time and ranged from 0.5% at 4 Weeks after Transplanting (WAT) to 47.2% at 14 WAT (Fig. 2). Anloga recorded the highest symptom severity of 42.7 and 47.2% at 12 and 14 WAT respectively. On the other hand, cv. Sunny F1 recorded the least symptom severity throughout the

whole period of study except at 14 WAT when cv. Arhcard had the least. At 6 WAT, there was no significant difference in symptom severity between cvs. Archard and Sunny F1 but both were significantly different from cv. California Wonder. However, at 8 WAT, a significant difference was observed between cv. Sunny F1 and California Wonder. Symptom severity among the cultivars was not significantly different at 4, 10, 12 and 14 WAT. Differences in the pattern and degree of symptom severity among the cultivars could be attributed to differences in genetic make-up of the cultivars as well as the strain of the virus and possible co-infection with other viruses (Sherwood *et al.*, 1986).

Disease Incidence (DI) among the various pepper cultivars showed a trend similar to that of symptom severity (Fig. 3). It was highest in cv. California Wonder from 6 WAT (26.4%) to 14 WAT (61.3%).

Table 1: DAS-ELISA detection of CMV, PVMV, PMMV and TMV in leaf extracts of nine pepper cultivars

Pepper cultivar	No. of samples tested	Type of virus identified and the number of samples infected											
		CMV	PVMV	PMMV	TMV	CMV+PVMV	CMV+PMMV	CMV+TMV	PMMV+PVMV	PVMV+TMV	PMMV+TMV	CPT	CPTM
Anloga	24	24	24	0	2	24	0	2	0	2	0	2	0
Antillas	24	14	20	0	2	6	0	2	0	2	0	2	0
Archard	24	16	24	0	2	16	0	2	0	2	0	2	0
Big sun	24	6	16	0	6	4	0	2	0	2	0	2	0
Bomb.	24	12	20	0	4	8	0	4	0	4	0	4	0
Calif. wonder	24	14	12	0	6	12	0	2	0	0	0	0	0
Forever F1	24	18	12	0	0	12	0	0	0	0	0	0	0
Legon 18	24	20	20	8	8	18	8	8	8	8	8	8	8
Sunny F1	24	14	22	0	0	14	0	0	0	0	0	0	0

CPT: CMV; PVMV, TMV and CPTM: CMV, PVMV, TMV and PMMV

Sunny F1 had the lowest DI at bi-weekly sampling periods, except at 14 WAT, when cv. Big Sun recorded the least incidence (18.5%). There were significant differences in disease incidence among the pepper cultivars at all the sampling periods except at 4 WAT.

Using antisera to the different viruses in a DAS-ELISA test, CMV, PVMV, PMMoV and TMV were all detected among the cultivars, with each cultivar infected by at least one of the four viruses (Table 1). CMV and PVMV were the most common viruses detected. All symptomatic samples of cvs. Archard and Anloga were found to be infected by PVMV. This virus has been reported in several West African countries where disease incidences as high as 100% have frequently been observed (Atiri and Dele, 1985). Similarly, CMV was detected in all symptomatic samples of Anloga, followed by Legon 18 (83.3%) and Forever F1 (75%). The high incidence of PVMV and CMV observed in this study is in agreement with Arogundade *et al.* (2012) who recorded an incidence in the range of 55.0 to 87.50% for PVMV and CMV in single as well as mixed infections on pepper during a survey in Nigeria. The high incidence of these two viruses in this study might be due to a number of factors such as proximity of the pepper plants to alternative hosts, climatic conditions, low quality seed and vector activity as reported by Shah *et al.* (2009).

Although TMV was found to be present in seven out of the nine cultivars, it was detected in few samples in each cultivar, with Legon 18 recording the highest infection (33.3%). Interestingly, cv. California Wonder, which has been labeled as resistant to TMV, was found to be infected by the virus (25%). It is possible that the plants may have been infected by a different strain of the virus that is found locally. PMMoV was detected only in symptomatic plants of cv. Legon 18 (33.3%).

Cases of mixed infections of two or more viruses were also observed. The most common was the co-infection by PVMV and CMV which occurred in 100% of samples of cv. Anloga, 75% of cv. Legon 18, 66.6% of cv. Archard, 58.3% of cv. Sunny F1 and 50% of cv.

California wonder with cv. Big Sun recording the least (16.7%) Co-infection of pepper by PVMV and CMV has also been reported elsewhere (Hameed *et al.*, 1995). Mixed infection of all four viruses was found in

cv. Legon 18. This variety was bred in Ghana and has been cultivated as far back as 1981 (Amoatey, 1981). The high rate of infection observed in this cultivar could be due to recycling of seeds which may have resulted in the accumulation of viruses.

CONCLUSION

Viral diseases are considered to be a major limiting factor in pepper production in Ghana, causing serious losses. In the present study, both local and exotic pepper cultivars were found to be susceptible, with each cultivar infected by at least one of the four viruses tested. Mixed infections involving two or more viruses were also recorded. CMV and PVMV were identified as the most common viruses, occurring in all the cultivars. These findings have revealed the need for a nationwide survey to monitor disease pattern of these viruses, assess the crop condition as well as collecting information regarding their prevalence, distribution and occurrence. The outcome of such a study would serve as a guide in management strategies to minimize crop losses and ensure sustainable production of pepper. Furthermore, the findings have necessitated the need for the production and use of virus-free seed by pepper farmers, breeding for virus resistance and adoption of efficient seed certification systems as well as strict quarantine measures to prevent the introduction of viruses especially PMMV to other areas both within and outside the country where this virus is not known to occur.

ACKNOWLEDGMENT

The Authors wish to thank the Cocoa Research Institute of Ghana (CRIG) for logistical support. We are also grateful to the Radiological and Medical Science Research Institute (RAMSRI), of the Ghana Atomic Energy Commission for the use of laboratory facilities.

REFERENCES

- Agrios, G.N., 2005. Plant Pathology. 5th Edn., Academic Press, Burlington, pp: 992, ISBN: 0120445654.

- Amoatey, H.M., 1981. Effect of age of transplants on performance of hot pepper (*Capsicum frutescens* L) in the Accra plains. B.Sc. Thesis, University of Ghana, Legon, pp: 30.
- Arogundade, O., S.B. Olusegun and T.K. Kareem, 2012. Occurrence and distribution of pepper veinal mottle virus and cucumber mosaic virus in pepper in Ibadan, Nigeria. *Virol. J.*, 9: 79.
- Atiri, G.I., 1992. Progress of *Pepper veinal mottle virus* disease in *Capsicum* peppers. *Crop Prot.*, 11(3): 255-259, DOI: 10.1016/0261-2194(92)90046-8.
- Atiri, G.I. and H.W. Dele, 1985. Pepper veinal mottle virus infection, host reaction, yield and aphid transmission in pepper plants. *Trop. Agr.*, 62: 190-192.
- Clark, M.F. and A.N. Adams, 1977. Characteristics of the micro plate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. General Virol.*, 34: 475-483.
- Gafalla, G.A., 2003. Plant virology in sub-Saharan Africa. Proceedings of a Conference Organized by IITA: 4-8 June, 2001, Hughes, J.A. and B.O. Odu (Eds.), International Institute of Tropical Agriculture, Ibadan, Nigeria. IITA, Ibadan, pp: 589, ISBN: 9781312149.
- González-Pérez, J.L., M.C. Espino-Gudiño, I. Torres-Pacheco, R.G. Guevara-González, G. Herrera-Ruiz and V. Rodríguez-Hernández, 2011. Quantification of virus syndrome in chili peppers. *Afr. J. Biotech.*, 10(27): 5236-5250.
- Green, S.K. and J.S. Kim, 1991. Characteristics and control of viruses infecting pepper: A literature review. Asian Vegetable Research and Development Center, pp: 60, ISBN: 9290580453.
- Green, S.K. and J.S. Kim, 1994. Sources of resistance to viruses of pepper (*Capsicum* spp.): A catalog. Asian Vegetable Research and Development Center, Taipei, pp: 64, ISBN: 9290580897.
- Hameed, S., H. Shah, H. Ali and S. Khalid, 1995. Prevalence of chilli viruses in Pakistan. Proceeding of the 5th National Congress of Plant Sciences, March 28-30, NARC. Islamabad.
- Jin, R., J. Pan, H. Xie, B. Zhou and X. Xia, 2009. Separation and quantitative analysis of capsaicinoids in chili peppers by reversed-phase argentation LC. *Chromatographia*, 70: 5-6.
- Millennium Development Authority, 2010. Investment opportunity in Ghana chilli pepper production. A publication of MiDA in conjunction with the United States Millennium Challenge Corporation, Retrieved from: <http://mida.gov.gh/site/wp-content/uploads/2010/07/Ghana-Chili-BOM-Final-Version.pdf>.
- Ochoa-Alejo, N. and R. Ramírez-Malagón, 2001. In Vitro Chili Pepper Biotechnology: *In Vitro* cell. *Dev. Biol. Plant.*, 37: 701-729.
- Piper, J.K., M.K. Handley and P.A. Kulakow, 1996. Incidence and severity of viral disease symptoms on eastern gamagrass within monoculture and polycultures. Elsevier. *Agric. Ecosyst. Environ.*, 59: 139-147.
- Rashid, M.H., K.M. Khalequzzaman, M.S. Alam, S.A. Uddin and S.K. Green, 2007. Screening of different sweet pepper lines against cucumber mosaic virus and chili veinal mottle virus. *Int. J. Sustain. Crop Prod.*, 2(3): 1-4.
- Shah, H., T. Yasmin, M. Fahim, S. Hameed and M.I. Haque, 2009. Prevalence, occurrence and distribution of chilli veinal mottle virus in Pakistan. *Pak. J. Bot.*, 41(2): 955-965.
- Sherwood, J.L., B.B. Reddick and K.E. Conway, 1986. Reaction of Bahamian hot pepper to single and double infection with Tobacco Mosaic Virus (TMV) and Potato Virus Y (PVY). *Phytopathology*, 76: 1126. (Abstr).