OPEN ACCESS

African Journal of Plant Science

November 2023
ISSN 1996-0824
DOI: 10.5897/AJPS
www.academicjournals.org

ACADEMIC JOURNALS
expand your knowledge

About AJPS

The African Journal of Plant Science (AJPS) is a peer reviewed open access journal. The journal commenced publication in September 2007. The African Journal of Plant Science covers all areas of plant science such as phytopathology, plant morphology, sustainable plant production, plant pathology and plant microbe biology.

Indexing

AgBiotechNet, Agricultural Economics Database, Agroforestry Abstracts, Animal Breeding Abstracts, Animal Production Database, Animal Science, Biofuels Abstracts, Botanical Pesticides, CAB Abstracts, CABI's Global Health Database, Chemical Abstracts (CAS Source Index - CASSI), CNKI Scholar, Crop Physiology Abstracts, Crop Science Database, Environmental Impact, Environmental Science Database, Field Crop Abstracts, Forest Science Google Scholar, Grasslands and Forage Abstracts, Helminthological Abstracts, Horticultural Science, Horticultural Science Abstracts, Irrigation and Drainage Abstracts, Maize Abstracts, Microsoft Academic, Nematological Abstracts, Nutrition Abstracts and Reviews Series A: Human and Experimental, Nutrition Abstracts and Reviews Series B: Livestock Feeds and Feeding, Nutrition and Food Sciences, Ornamental Horticulture, Parasitology Database, Plant Breeding Abstracts, Plant Genetic Resources Abstracts, Plant Genetics and Breeding Database, Plant Growth Regulator Abstracts, Plant Protection Database, Potato Abstracts, Poultry Abstracts, Protozoological Abstracts, Rice Abstracts, Rural Development Abstracts, Seed Abstracts, Soil Science Database, Soils and Fertilizers Abstracts, Soybean Abstracts, Sugar Industry Abstracts, The Essential Electronic Agricultural Library (TEEAL), Veterinary Science Database, VetMed Resource, Weed Abstracts, Wheat, Barley and Triticale Abstracts, World Agricultural Economics and Rural Sociology Abstracts

Open Access Policy

Open Access is a publication model that enables the dissemination of research articles to the global community without restriction through the internet. All articles published under open access can be accessed by anyone with internet connection.

The African Journal of Plant Science is an Open Access journal. Abstracts and full texts of all articles published in this journal are freely accessible to everyone immediately after publication without any form of restriction.

Article License

Article Copyright

When an article is published by in the African Journal of Plant Science, the author(s) of the article retain the copyright of article. Author(s) may republish the article as part of a book or other materials. When reusing a published article, author(s) should; Cite the original source of the publication when reusing the article. i.e. cite that the article was originally published in the African Journal of Plant Science. Include the article DOI Accept that the article remains published by the African Journal of Biotechnology (except in occasion of a retraction of the article). The article is licensed under the Creative Commons Attribution 4.0 International License.

A copyright statement is stated in the abstract page of each article. The following statement is an example of a copyright statement on an abstract page.
Copyright ©2016 Author(s) retains the copyright of this article.

Self-Archiving Policy

The African Journal of Plant Science is a RoMEO green journal. This permits authors to archive any version of their article they find most suitable, including the published version on their institutional repository and any other suitable website.
Please see http://www.sherpa.ac.uk/romeo/search.php?issn=1684-5315

Digital Archiving Policy

The African Journal of Plant Science is committed to the long-term preservation of its content. All articles published by the journal are preserved by Portico. In addition, the journal encourages authors to archive the published version of their articles on their institutional repositories and as well as other appropriate websites.
https://www.portico.org/publishers/ajournals/

Metadata Harvesting

The African Journal of Plant Science encourages metadata harvesting of all its content. The journal fully supports and implement the OAI version 2.0, which comes in a standard XML format. See Harvesting Parameter

Memberships and Standards

Academic Journals strongly supports the Open Access initiative. Abstracts and full texts of all articles published by Academic Journals are freely accessible to everyone immediately after publication.

All articles published by Academic Journals are licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0). This permits anyone to copy, redistribute, remix, transmit and adapt the work provided the original work and source is appropriately cited.

Crossref is an association of scholarly publishers that developed Digital Object Identification (DOI) system for the unique identification published materials. Academic Journals is a member of Crossref and uses the DOI system. All articles published by Academic Journals are issued DOI.

Similarity Check powered by iThenticate is an initiative started by CrossRef to help its members actively engage in efforts to prevent scholarly and professional plagiarism. Academic Journals is a member of Similarity Check.

CrossRef Cited-by Linking (formerly Forward Linking) is a service that allows you to discover how your publications are being cited and to incorporate that information into your online publication platform. Academic Journals is a member of CrossRef Cited-by.

Academic Journals is a member of the International Digital Publishing Forum (IDPF). The IDPF is the global trade and standards organization dedicated to the development and promotion of electronic publishing and content consumption.

Contact

Editorial Office: ajps@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/AJPS

Submit manuscript online http://ms.academicjournals.org

Academic Journals
73023 Victoria Island, Lagos, Nigeria
ICEA Building, 17th Floor,
Kenyatta Avenue, Nairobi, Kenya.

Table of Content

Vol. 17(10), pp. 93-99, November 2023
DOI: 10.5897/AJPS2023.2325
Article Number: F972F4771499
ISSN 1996-0824

http://www.academicjournals.org/AJPS

ACADEMIC JOURNALS
expand your knowledge

African Journal of Plant Science

Full Length Research Paper

An investigation of the photophysiology and phenology of cultivated African violet plants

O. Roger Anderson

Biology and Paleo Environment, Lamont-Doherty Earth Observatory, Columbia University, Palisades, New York, 10964, USA.

Received 16 September, 2023; Accepted 30 October, 2023

African violets (*Streptocarpus ionanthus*), originally native to the Eastern Arc Mountains of Africa and adjacent submontane or montane forests in Kenya and Tanzania, are commonly grown as ornamental plants and have been extensively studied by horticulturists. However, there is a lack of laboratory data regarding their ecophysiology. Given the growing interest in conserving African violets in their threatened natural habitats, it would be beneficial to establish baseline data on their physiological ecology, helping to document their response to changing environmental conditions in their natural environment. To address this need, we conducted an analysis of the net photosynthesis rate, respiration rate, and leaf fluorescence of mini and standard strains of African violet plants within a controlled laboratory setting. Additionally, we examined variations in the photosynthesis rate, respiration rate, and leaf fluorescence data for leaves of different ages (phenology) in the standard strain. The results showed that the mean net photosynthesis rate in the older whorl of leaves was approximately 50% of the rate observed in the younger leaves. Furthermore, the mature leaf respiration rate accounted for about 25 to 30% of the net photosynthesis. This particular metric, the ratio of respiration to net photosynthesis, is significant in estimating the carbon balance of the plants. It helps us understand how much carbon (C) is gained through photosynthesis compared to the amount of carbon lost through respiration.

Key words: Environmental carbon balance, leaf relative chlorophyll content analysis, leaf chlorophyll fluorescence analysis, photosynthesis rate, respiration rate, specific leaf area.

INTRODUCTION

African violets are among the most commonly cultivated ornamental plants in households and conservatories. From a taxonomic perspective, they belong to the Gesneriaceae family and were originally classified as *Saintpaulia ionantha* H. Wendl. However, based on molecular genetic analysis, African violets were reclassified under the *Streptocarpus* genus and renamed *Streptocarpus ionanthus* (H. Wendl.) Christenh (Christenhusz, 2012). Furthermore, there has been a growing interest in the molecular phylogenetics of *S. ionantha*, including the examination of phylogenetic relationships among various subspecies (Kyalo et al., 2002). African violet species are native to the Eastern Arc Mountains of Africa and are most prominently found in

E-mail: ora@ldeo.columbia.edu.

Figure 1. Laboratory cultured African violets. Standard plant (left) and mini plant (right). Marker = 6 cm.
Source: Author.

the submontane or montane forests of Kenya and Tanzania. These regions are recognized as one of the 25 global biodiversity hotspots (Myers, 1988). Interestingly, a few African violet species are also found in lowland forests and have played a significant role as the primary source of species from which all horticultural cultivars have been developed (Clarke, 1998). Over the last century, African violets have been intensively cultivated, and new varieties have been bred through selective breeding, resulting in thousands of cultivated varieties that are mass-produced by the horticulture industry (Baatvik, 1993).

However, concerns have been growing regarding the conservation status of African violets in their natural habitat (Eastwood et al., 1998). To gain a comprehensive understanding of the environmental and anthropogenic factors that may pose a threat to the conservation of African violet species, additional research is needed to explore their physiological ecology. This research can provide evidence of their adaptive features, which are crucial for monitoring and documenting their status in their natural environment. It is worth noting that while considerable attention has been given to the horticultural aspects of their physiology, propagation, and growth (Faust and Heins, 1993; Yun et al., 1997; Boschi et al., 2000; Streck, 2004; Dewir et al., 2015; Teixeira et al., 2016; Ahamadi et al., 2023; Akbarian et al., 2023), there is only a modest representation of published laboratory research on their ecophysiology.

MATERIALS AND METHODS

Culture conditions and sampling methods

Mature African violet plants, including both mini and standard strains, were sourced from commercial plant distributors, specifically Violet Barn in Naples, New York, and Valli Florist in New York City, New York, USA (Figure 1). These African violet plants were cultivated in plastic pots filled with commercially available potting soil formulated for African violets, specifically using Scotts Miracle-Gro from Marysville, Ohio, USA.

The plants were carefully nurtured in an environmentally controlled culture room at the Lamont-Doherty Earth Observatory of Columbia University. The growing conditions included a temperature of 25°C and a light cycle of 14 h of light followed by 10 h of darkness. The light was provided using fluorescent illumination with an intensity of 100 µmol photons m^{-2} s^{-1}, and the relative humidity was maintained at 60%.

Each mature plant, whether of the mini or standard strain, had approximately four whorls of leaves. Individual mature leaves were collected in the morning and promptly transported to the laboratory within the same building for subsequent physiological analyses. Specifically, ten plants of the mini strain and ten plants of the standard strain were analyzed for their leaf morphology, photosynthesis rate, and respiration rate.

For the phenology study, leaves of the standard African violet plants were the focus of analysis. One leaf was collected from each of the four whorls present in the rosette of leaves from a total of six plants, resulting in a total of twenty-four leaves for physiological analyses. The youngest leaves were collected from the central whorl at the growing apex, while the oldest leaves were obtained from the outer peripheral whorl.

Morphology

African violet mean leaf thickness (mm) was assessed based on 40 leaf measurements of four plants, each of the mini and standard African violet plants, using a Micro-precision digital caliper (0.01 mm precision) manufactured by *iGaging* (San Clemente, California, USA). The mean leaf area (cm^2) was based on 10 sampled leaves from each strain using a Leaf Area Meter (Model AM-350, Opti-Sciences, Inc., Hudson, New Hampshire, USA).

Leaf physiological and phenological analyses

The following variables were examined to document physiological

and phenological characteristics of the leaves:

1. Leaf chlorophyll fluorescence including evidence of quantum efficiency expressed as a ratio of variable fluorescence to maximum fluorescence (Fv/Fm) and electron transfer per reaction center beyond the quinone intermediate (Q_A) in the electron-transport chain (ET_0/RC).
2. Leaf chlorophyll content index (CCI), a relative index measuring chlorophyll content per unit leaf area, and the specific leaf area (SLA), a measure of the ratio of leaf area to leaf dry mass.
3. Leaf net photosynthesis rate and dark respiration rate.

Understanding the relative rates of photosynthesis, respiration, growth, and carbon storage in vegetation is of paramount importance (Atkin et al., 2006). Such an analysis is crucial for gaining insights into the overall growth and carbon storage in plants. This study employs appropriate methods to obtain comparative data on net photosynthesis and respiration rates in both mini and standard strains of African violets. The goal is to incorporate this data into a broader analysis of the role of African violets in the carbon balance relative to their environment, particularly in the context of CO_2-based carbon balance. The net photosynthesis rate of the leaves was assessed using an infra-red gas analyzer (IRGA) system; specifically, model BTA from Vernier, Beaverton, Oregon. The system featured an optically clear, 163 cm^3 assay chamber and was illuminated using a Light Emitting Diode (LED) source. The relative humidity within the sample cuvette was maintained within the range of 85 to 90% to reduce excessive vapor pressure deficit. The CO_2 concentration in the assay cuvette was set to match the ambient atmospheric concentration, which was 417 ppm. The measurement of each leaf took approximately 10 min.

Mean photosynthesis rates were assessed for both the mini and standard strains at four different light intensities, namely 10, 25, 50, and 100 µmol m^{-2} s^{-1}. These rates were measured and expressed in three different units: (1) based on leaf area (µmol m^{-2} s^{-1}), (2) based on fresh weight (FW) (nmol g FW^{-1} min^{-1}), and (3) based on dry weight (DW) (µmol g DW^{-1} min^{-1}). The mean respiration rate was determined at a constant temperature of 25°C using the same apparatus. The assay chamber was completely darkened, and the results were expressed in the same three units: per leaf area, leaf fresh weight, and leaf dry weight. The leaf sample was kept in a dark condition within the respiration cuvette until the reaction centers of the photosystems in the leaves reached equilibrium with the darkened state (approximately 2 min). Measurements were initiated when a steady state respiration rate was achieved.

Each leaf sample's chlorophyll concentration index (CCI), expressed in mg m^{-2} (Gitelson et al., 1999), and was obtained using a CCM-300 chlorophyll content meter (Opti-Sciences, Inc., Hudson, New Hampshire). At least ten measurements were made for each leaf sampled. The mean value, obtained from the ten measurements of leaves on one plant, was used to calculate the overall mean CCI value for a given plant. An OS-30p+ Chlorophyll Fluorometer (Opti-Sciences, Inc., Hudson, New Hampshire) was used to obtain the leaf fluorescence data. This included two assays based on the JIP test application in the OS-30p+ instrument: 1) leaf quantum yield efficiency expressed as variable fluorescence/ maximum fluorescence (Fv/Fm), and 2) evidence of electron transport per reaction center (ET_0/RC) from photosystem II (PS II) to the quinone intermediate (Q_A) and beyond in the electron-transport chain. Leaf samples were dark adapted for 20 to 30 min. Before the measurements were made to ensure that the reaction centers (RC) had come to equilibrium with the darkened state. Following the leaf fluorescence assay, the area of the leaf, expressed as cm^2, was assessed using a Leaf Area Meter (Model AM-350, Opti-Sciences, Inc., Hudson, New Hampshire, USA). After drying overnight at 60°C in a laboratory oven, the leaf's fresh weight and the dry weight were determined using a Sartorius digital balance. This data was also used to calculate the mean specific leaf area (leaf area in cm^2/dry weight in g) (Wolf et al., 1972).

All results of the assays are presented as the mean ± standard error of the mean (SEM) calculated using an Excel spreadsheet (Microsoft, Inc., Redmond, Washington State, USA). A t-Test (GraphPad Software, Boston, Massachusetts, USA) was used to test the statistical significance of mean differences for data presented in each of the Tables 1 to 4. The criterion level of significance was set at $p \leq 0.05$. A Kolmogorov-Smirnov test was used to verify that the data for each t-test analysis was sufficiently normally distributed to apply the parametric t-test.

RESULTS

Morphology

The mean leaf area (cm^2) based on 10 sampled leaves from each strain was 7.7 ± 0.4 for the mini strain and 17.1 ± 1.6 for the standard strain. The difference in means is statistically significant ($t = 5.70$, $p < 0.01$, $N = 10$). The mean leaf lamina thickness for the mini strain African violet plants (0.99 ± 0.03 mm) was considerably thinner than for the standard strain (1.55 ± 0.04 mm) ($t = 11.2$, $p < 0.01$, $N = 10$). In both strains, leaf shape varied from ovate to cordate.

Physiology and phenology

Table 1 displays the mean net photosynthesis rates for both the mini strain and standard strain African violets. The data shows that there is no statistically significant difference in the photosynthesis rates between the two strains when expressed per unit area, per unit fresh weight, or per unit dry weight. This is evident from the comparable mean values and the results of t-tests, which confirm the lack of a significant difference.

Table 2 presents the mean dark respiration rates for the two strains, along with the results of statistical tests included in the table's footnote. The data indicates that the mean values are quite similar for both strains, and the statistical results do not show a significant difference. It is worth noting that even though the two strains differ substantially in plant size, leaf thickness, and leaf area (as shown in Figure 1), their mean respiration rates are not significantly different.

Given the similar leaf physiological properties observed in Table 2 for the African violet mini and standard strains, leaf phenology analyses were only conducted on the standard African violet strain. This strain is the most commonly cultivated and has larger leaves that are more suitable for laboratory analysis.

Table 3 presents mean data for leaf fluorescence (Fv /Fm, ET_0/RC), chlorophyll concentration index (CCI), and specific leaf area (SLA) from each of the four whorls of leaves in six plants. The mean values for the fluorescence leaf data (Fv /Fm and ET_0/RC) are statistically significant, with higher values observed in the younger leaves of whorl 1 compared to the older leaves in whorl 4. However,

Table 1. Mean ± SEM net photosynthesis rate for the mini and standard African violet strains[a].

Strain	Photosynthesis rate		
	(μmol CO_2 m^{-2} s^{-1})[b]	(nmol CO_2 g_{FW}^{-1} min^{-1})[c]	(μmol CO_2 g_{DW}^{-1} min^{-1})
Mini strain			
10	0.27 ± 0.04	22.0 ± 4.14	0.59 ± 0.11
25	0.59 ± 0.07	48.0 ± 6.97	1.27 ± 0.21
50	0.78 ± 0.10	64.9 ± 10.59	1.70 ± 0.28
100	0.94 ± 0.10	76.9 ± 10.36	1.98 ± 0.25
Standard strain			
10	0.27 ± 0.02	21.9 ± 1.51	0.52 ± 0.06
25	0.57 ± 0.04	46.2 ± 4.66	1.15 ± 0.10
50	0.83 ± 0.05	67.3 ± 6.02	1.67 ± 0.13
100	0.96 ± 0.06	78.4 ± 7.40	1.91 ± 0.16

[a]N = 10 plants measured for each mean value. Light intensity expressed as photosynthetic photon flux density (PPFD) in units of μmol photons m^{-2} s^{-1}, and mean leaf photosynthesis rate expressed per leaf area (m^{-2}), per leaf fresh weight in grams (g FW^{-1}), and per leaf dry weight in grams (g DW^{-1}). Means for each of the three columns of data comparing the mini and standard strains are substantially comparable, and not significantly different. [b](t = 0.17, p = 0.86), [c](t = 0.08, p = 0.94), [d](t = 0.42, p = 0.68).

Table 2. Mean ± SEM Leaf respiration rate expressed per leaf area (m^{-2}), per leaf fresh weight in grams (g FW^{-1}), and per leaf dry weight in grams (g DW^{-1}) for the mini and standard African violet strains[a].

Strain	(μmol CO_2 m^{-2} s^{-1})[b]	(nmol CO_2 g_{FW}^{-1} min^{-1})[c]	(μmol CO_2 g_{DW}^{-1} min^{-1})[d]
Mini strain	0.18 ± 0.02	23.77 ± 8.78	0.38 ± 0.03
Standard strain	0.24 ± 0.03	20.59 ± 3.78	0.48 ± 0.07

[a] N = 10 plants measured for each mean value. Mean values for the mini and standard strains are substantially comparable, and not significantly different. [b](t = 1.68, p = 0.11), [c](t = 0.33, p = 0.74), [d](t = 1.31, p = 0.21).

Table 3. Standard African violet leaf phenology means ± SEM for fluorescence data (F_v /F_m, ET_0/RC), chlorophyll concentration index (CCI), and specific leaf area (SLA)[a].

Whorl	F_v /F_m[b]	ET_0/RC[c]	CCI (mg m^{-2})[d]	SLA[e]
1	0.75 ± 0.01	1.34 ± 0.06	11.60 ± 1.37	305.71 ± 1.04
2	0.71 ± 0.02	1.31 ± 0.05	10.30 ± 0.39	278.45 ± 21.25
3	0.72 ± 0.02	1.23 ± 0.04	12.83 ± 0.89	293.07 ± 17.05
4	0.62 ± 0.05	1.14 ± 0.06	8.34 ± 1.34	335.79 ± 14.82

[a]N = 6 plants measured for each mean value per whorl. Statistical comparison of means for whorl 1 and whorl 4: [b](t = 2.55, p = 0.03), [c](t = 2.36, p = 0.04), [d](t = 1.7, p = 0.12), [e](t = 2.02, p = 0.07).

the mean values for CCI and SLA are not statistically different when comparing whorl 1 leaves to whorl 4 leaves.

Table 4 displays the variation in mean photosynthesis rates and mean respiration rates across the four whorls of leaves in six plants. It appears that both the mean photosynthesis rate and mean respiration rate tend to be higher in the younger leaves of whorl 1 compared to the older leaves of whorl 4. However, when subjected to t-test analysis for comparisons of means between whorl 1 and whorl 4, only the photosynthesis mean rates show a statistically significant difference between the two whorls. This significance is evident for each of the three column variables (μmol CO_2 m^{-2} s^{-1}, nmol CO_2 g FW^{-1} min^{-1}, and μmol CO_2 g DW^{-1} min^{-1}). In contrast, the differences in mean respiration rates are not statistically significant.

DISCUSSION

Leaf morphology

As a context for this study, the leaf morphology (mean area and mean thickness) of the mini and standard

Table 4. Standard African violet leaf phenology: net photosynthesis rate ± SEM and respiration rate ± SEM for leaves in whorls 1 to 4[a].

Whorl	Photosynthesis rate		
	(µmol CO_2 m^{-2} s^{-1})[b]	(nmol CO_2 g_{FW}^{-1} min^{-1})[c]	(µmol CO_2 g_{DW}^{-1} min^{-1})[d]
1	0.94 ± 0.11	72.1 ± 10.05	1.63 ± 0.19
2	0.78 ± 0.10	52.7 ± 6.91	1.34 ± 0.23
3	0.71 ± 0.11	50.2 ± 8.39	1.27 ± 0.21
4	0.42 ± 0.12	33.0 ± 10.62	0.81 ± 0.20
	Respiration rate		
	(µmol CO_2 m^{-2} s^{-1})[e]	(nmol CO_2 g_{FW}^{-1} min^{-1})[f]	(µmol CO_2 g_{DW}^{-1} min^{-1})[g]
1	0.28 ± 0.07	21.7 ± 5.84	0.52 ± 0.14
2	0.24 ± 0.08	15.2 ± 5.0	0.39 ± 0.11
3	0.22 ± 0.05	15.2 ± 3.78	0.38 ± 0.09
4	0.11 ± 0.03	7.8 ± 2.42	0.20 ± 0.05

[a]N = 6 plants measured for each mean value per whorl. The photosynthetic photon flux density (PPFD) for photosynthesis measurements was 100 µmol m^{-2} s^{-1}, equivalent to the light intensity in the environmentally controlled culture chamber. Statistical t-test results for each column of data are as follows: [b](t = 3.16, p = 0.01); [c](t = 2.67, p = 0.02); [d](t = 3.93, p = 0.003); [e](t = 2.16, p = 0.06); [f](t = 2.20, p = 0.052); [g](t = 2.15, p = 0.057).

African violet plants was examined. As may be expected, the mini strain had thinner leaves and smaller leaf area than the standard strain. A search of the literature suggests that this is the first report of these comparative data for leaf morphology in African violets. However, there have been substantial studies of variations of leaf morphology related to experimental treatments such as growth substrate (Ghehsareh et al., 2023), effects of light spectral quality on leaf area and thickness (Ahamadi et al., 2023), and effects of adding UV light in the visual spectrum on leaf expansion (Akbarian et al., 2023), including modelling studies of growth (Faust and Heins, 1993; Streck, 2004).

Photophysiology and phenology

A careful review of the literature indicated that few prior research studies have examined the photosynthesis rate of African violets in relation to light intensity. However, Dewir et al. (2015) reported that pot-grown plantlets of African violets exhibited maximum net photosynthesis at a moderate light intensity of 70 µmol m^{-2} s^{-1} when compared with either a lower or higher intensity (35 and 100 µmol m^{-2} s^{-1}, respectively). The results reported here with mature mini and standard strains of African violets showed net photosynthesis was highest at a light intensity of 100 µmol m^{-2} s^{-1} across a light intensity range of 10 to 100 µmol m^{-2} s^{-1}, with a mean assimilation rate of c. 2.0 µmol CO_2 g_{DW}^{-1} min^{-1} at the highest light intensity. Based on this evidence, the carbon assimilation rate, when illuminated with a light intensity of 100 µmol m^{-2} s^{-1} is c. 23 µg of C g_{DW}^{-1} min^{-1}. During a photoperiod of 12 h illumination, assuming a steady rate of photosynthesis, the gain in carbon during the 12-h photoperiod would be 16.5 mg C per g dry weight of the leaf. In general, a careful search of the literature indicated that very little, if any, research has been done on the photophysiology of species in the Gesneriaceae family. Given their significance in the natural environment and their horticultural value, more research is warranted to elucidate the ecophysiology of this widely occurring family of plants.

Using leaf fluorescence analysis, Dewir et al. (2015) reported that F_v/F_m values in African violet plantlets varied inversely, with light intensity being highest (F_v/F_m = 0.80) at a light intensity of 35 µmol m^{-2} s^{-1} and lowest (0.75) at light intensity of 100 µmol m^{-2} s^{-1}. In the present study, F_v/F_m was examined for leaves of varying maturity when grown at a PPFD of 100 µmol m^{-2} s^{-1}. The mean F_v/F_m values varied from 0.68 for older leaves in whorl 4 to 0.75 for young leaves in whorl 1. This value for young leaves (0.75) is close to a value of 0.80 reported by Dewir et al. (2015) for the young leaves of African violet plantlets. The mean net photosynthesis rate in the current study also decreased with increasing maturity of the leaves in the four whorls of standard African. For example, the net mean photosynthesis rate (µmol CO_2 assimilated m^{-2} s^{-1}) when assessed at a light intensity of 100 µmol m^{-2} s^{-1} was 0.94 ± 0.11 for young leaves (whorl 1) and 0.42 ± 0.12 for older leaves (whorl 4). Prior research with a wide variety of angiosperms has shown a similar decline in photosynthesis rate with leaf age, including studies of wheat (Suzuki et al., 1987), grape vines (Kriedemann, 1988), conifer trees (Freeland, 1952), and tropical canopy trees (Kitajima et al., 1997). The

mean dark respiration rate in the current study was not significantly higher for whorl 1 compared to whorl 4 leaves, although there was a statistically significant difference in the photosynthesis rate (Table 4). In general, the results indicated that respiration rates were not significantly different for any of the leaf variables examined in this study. Thus, in general, respiration rate appeared to be less variable compared to photosynthesis rates for the experimental conditions used in this research.

Overall, the respiration rate is approximately 25 to 30% of the net photosynthesis rate. Based on prior experimental research and modelling studies of plants, some variation in the ratio of respiration to photosynthesis can be expected depending on the species, temperature of growth, and the state of plant growth (Atkin et al., 2006; Van Oijen et al., 2010). This metric (ratio of respiration to net photosynthesis) is important in estimating the carbon balance of the plants; that is, how much C is gained through photosynthesis relative to the amount of C lost through respiration, and has significance for estimating climate change related to atmospheric CO_2 concentrations (Heskel, 2018). It may be an important measure of African violet vitality in different locales in its natural habitat in Africa, where this species is incurring environmental stress or other threats to survival.

A thorough literature search revealed few prior research studies have reported the SLA for African violets. Ahamadi et al. (2023) published SLA data for African violet plants grown with different wavelengths of light, but they calculated the SLA based on units of cm^2 per g fresh weight rather than units of dry weight as most typically used. Akbarian et al. (2023) reported SLA values based on leaf dry weight when African violet plants were grown with illumination of varying wavelengths in combination with UV irradiation. They found SLA values in the range of 140 to 240. In the current study, the SLA values based on dry weight were 300 to 465 for standard African violet plants with a mean SLA of 360. Given substantial evidence that SLA is positively correlated with photosynthesis rate in a variety of angiosperms, especially when the photosynthesis rate is expressed per units of dry weight (Poorter and Evans, 1998; Reich et al., 1998), additional attention should be given to studying the effects of environmental variables on the SLA of African violet plants in the natural environment as well as in laboratory-based studies.

In the current research, the mean net photosynthesis rate was expressed in three ways: related to leaf area, per leaf fresh weight, and per leaf dry weight. The net photosynthesis rate expression per leaf area unit is a widely used metric. It is particularly useful in accounting for the physiological fate of solar energy absorbed in plant ecosystems because the amount of solar radiation absorbed per unit area of the environment can be quantitatively related to the amount of the incident radiation absorbed and utilized to drive CO_2 assimilation by photosynthesis per leaf area (either for individual plants or for stands of plants). However, expression of photosynthesis rates per unit of weight, particularly dry weight, is particularly useful in ecosystem carbon budget analyses because the amount of energy consumed to support carbon uptake by primary production can be related quantitatively to the total carbon biomass of a given plant or stand of plants in an ecosystem. Furthermore, as explained earlier, the best estimates of the prediction of photosynthesis rates based on SLA are obtained when the photosynthesis rate is expressed per unit of leaf dry weight (Poorter and Evans, 1998). With increasing interest in conserving African violets in the natural environment, it may be particularly important to make measurements of photosynthesis and respiration in the natural environment based on units of dry weight in addition to measurements based on units of leaf area. This will permit a more accurate accounting of the amount of gain or loss in carbon by the total biomass of plants in a given stand or regional ecosystem. This may be especially useful where changing environmental conditions may place the African violet plants at risk for survival, and a more accurate accounting of their C gain and loss is of importance in assessing their vitality.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

REFERENCES

Ahamadi L, Matloobi M, Motallebi-Azar A (2023). Light source spectrum influences long-term flowering cycles and visual appearance in African violet (*Saintpaulia ionantha* Wendl.). Lighting Research and Technology 20:1-18.

Akbarian B, Matloobi M, Motallebi-Azar A, Dadpour M (2023). The role of UV radiation added to LED lighting system in growth control of African violet (*Saintpaulia ionantha* Wendl.). Journal of Ornamental Plants 13:75-84.

Atkin OK, Scheurwater I, Pons TL (2006). Respiration as a percentage of daily photosynthesis in whole plants is homeostatic at moderate, but not high, growth temperatures. New Phytologist 174(2):367-380.

Baatvik ST (1993). The genus *Saintpaulia* (Gesneriaceae) 100 years: History, taxonomy, ecology, distribution and conservation. Fragmenta Floristica et Geobotanica Polonica, Supplement 2:97-112.

Boschi C, Di Benedetto A, Papayani P, Cremona C, Bendicto D (2000). Coverfilms and light quality responses in *Saintpaulia ionantha*. Acta Horticulturae 515:135-140.

Christenhusz MJM (2012). On African violets and Cape primroses–towards a monophyletic *Streptocarpus* (Gesneriaceae). Phytotaxa 46(1):3-9.

Clarke GP (1998). Plants in peril, 24: Notes on lowland African Violets (*Saintpaulia*) in the wild. Curtis's Botanical Magazine 15(1):62-67.

Dewir YH, El-Mahrouk M E-S, Al-Shmgani HS, Rihan HZ, Teixeira da Silva JA, Fuller MP (2015). Photosynthetic and biochemical characterization of in vitro-derived African violet (*Saintpaulia ionantha* H. Wendl) plants to ex vitro conditions. Journal of Plant Interactions 10:101-108.

Eastwood A, Bytebier B, Tye H, Tye A, Robertson A, Maunder M (1998). The conservation status of Saintpaulia. Curtis's Botanical Magazine 15(1):49-62.

Faust JE, Heins RD (1993). Modeling leaf development of the African

violet (*Saintpaulia ionantha* Wendl.). Journal of the American Horticultural Society 118(6):747-751.
Freeland RO (1952). Effects of age of leaves upon the rate of photosynthesis in some conifers. Plant Physiology 27(4):685-690.
Ghehsareh MG, Milani F, Reezi S, Fatahi M (2023). The effect of different types of culture substrate on some morphological and physiological indices of African violet (*Saintpaulia ionantha* H. Wendl). Plant Production 45(4):475-489.
Gitelson AA, Buschmann C, Lichtenthaler HK (1999). The chlorophyll fluorescence ratio F735/F700 as an accurate measure of chlorophyll content in plants. Remote Sensing of Environment 69:296-302.
Heskel MA (2018). Small flux, global impact: integrating the nuances of leaf mitochondrial respiration in estimates of ecosystem carbon exchange. American Journal of Botany 105(5):815-818.
Kitajima K, Mulkey SS, Wright SJ (1997). Decline of photosynthetic capacity with leaf age in relation to leaf longevities for five tropical canopy tree species. American Journal of Botany 84(5):702-708.
Kyalo CM, Li ZZ, Mkala EM, Malombe I, Hu GW, Wang QF (2002). The first glimpse of *Streptocarpus ionanthus* (Gesneriaceae) phylogenomics: Analysis of five subspecies' chloroplast genomes. Plants 9:456. doi:10.3390/plants9040456
Kriedemann PE (1988). Photosynthesis in vine leaves as a function of light intensity, temperature, and leaf age. Vitis 7(3):213-230.
Myers N (1988). Threatened biotas: ''Hots spots'' in tropical forests. Environmentalist 8:187-208.
Poorter H, Evans JR (1998). Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. Oecologia 116:26-37.
Reich PB, Ellsworth DS, Walters MB (1998). Leaf structure (specific leaf area) modulates photosynthesis-nitrogen relations: evidence from within and across species and functional groups. Functional Ecology 12(6):948-958.
Streck NA (2004). A temperature response function for modeling leaf growth and development of the African violet (Saintpaulia ionantha Wendl.) Ciencia Rural 34:55-62.
Suzuki S, Nakamoto H, Ku MSB, and Edwards GE (1987). Influence of leaf age on photosynthesis, enzyme activity, and metabolite levels in wheat. Plant Physiology 84(4):1244-1248.
Teixeira da Silva JA, Dewir, YH, Wicaksono A, Kher MM, Kim H, Hosokawa M, Zeng S (2016). Morphogenesis and developmental biology of African violet (*Saintpaulia ionantha* H. Wendl.). Journal of Plant Development 23:15-25.
Yun JG, Hayashi T, Yazawa S, Yasuda Y, Katoh T (1997). Degradation of photosynthetic activity of *Saintpaulia* leaf by sudden temperature drop. Plant Science 127:25-38.
Van Oijen M, Schapendonk A, Höglind M (2010). On the relative magnitudes of photosynthesis, respiration, growth and carbon storage in vegetation. Annals of Botany 103(5):793-797.
Wolf DD, Carson EW, Brown RH (1972). Leaf area index and specific leaf area determinations. Journal of Agronomy Education 1(1):24-27.

Vol. 17(10), pp. 100-107, November 2023
DOI: 10.5897/AJPS2023.2308
Article Number: 9842D4171515
ISSN 1996-0824

http://www.academicjournals.org/AJPS

ACADEMIC JOURNALS
expand your knowledge

African Journal of Plant Science

Full Length Research Paper

Occurrence and distribution of viruses associated with papaya ringspot disease in Kenya

Naomi Nzilani Mumo[1]*, Elijah Miinda Ateka[1], George Edward Mamati[1], Fredah Karambu Rimberia[1] and George Ochieng' Asudi[2]

[1]Depatment of Horticulture and Food Security, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 62000-00200, Nairobi, Kenya.
[2]Department of Biochemistry, Microbiology and Biotechnology Kenyatta University, P. O. Box 43844-00100, Nairobi, Kenya.

Received 29 March, 2023; Accepted 3 November, 2023

Papaya ringspot disease is a serious threat to papaya production in Kenya. For effective management, it is important to determine the occurrence and distribution of the viruses associated with the disease. A survey was conducted in 2017, covering a total of 103 papaya fields in major papaya production areas in the country. To determine the disease incidence, 20 plants per field were visually inspected for symptoms associated with the disease. Disease severity was evaluated on a scale of 1 to 5, while disease prevalence was determined as the proportion of fields showing disease symptoms per county expressed as a percentage. A total of 287 leaf samples were collected from surveyed fields and tested for Moroccan watermelon mosaic virus (MWMV), cowpea mild mottle virus (CpMMV), and papaya mottle-associated virus (PaMV) using polymerase chain reaction (PCR)-based techniques. The highest (71.4%) disease incidence was recorded in Kiambu County, while the lowest was recorded in Busia County (2.8%). No symptomatic plants were observed in Siaya and Bungoma (0%) counties. Disease prevalence ranged from 0 to 100%. The highest disease severity, 4.0, was reported in Baringo County; while the lowest, 2.0, was reported in Kwale, Kilifi, and Taita Taveta counties. MWMV was the most prevalent, with 140 out of 287 samples testing positive and also widespread, having been detected in 11 out of the 22 counties surveyed. PaMV was the second most prevalent, detected in 39 out of 287 samples collected and in 9 out of 22 counties. CpMMV was the least prevalent, detected in 7 out of 287 samples and in three counties. The occurrence of both MWMV and PaMV was detected in five counties, while the occurrence of PaMV and CpMMV was detected in three counties. The presence of MWMV, PaMV and CpMMV was detected in one county. Viruses associated with papaya ringspot disease in Kenya are widespread in papaya-growing regions, with some counties reporting 100% disease prevalence. The development and implementation of control strategies for the disease in the country are of paramount importance. In the future, it is important to identify factors influencing disease spread in the country for effective management.

Key words: Incidence, viral diseases, control strategies, farmers, interventions.

INTRODUCTION

Carica papaya L. is an important fruit crop in Kenya, grown by small and large-scale farmers for subsistence, local, and export markets. However, statistics regarding its production in the country are unsatisfactory. For instance, there has been a steady increase in the area of papaya production over recent years with no substantial increase in yields (HCDA, 2021). The low papaya yields are mostly attributed to poor agronomic practices, including the lack

of improved crop varieties and crop damage by pests and diseases (Rimberia and Wamocho, 2014; Kansiime et al., 2020; HCDA, 2021).

Viral diseases threaten plant crops by impairing their growth and vigor, leading to a decrease in gross yields. These diseases also reduce the quality of produce, thereby decreasing marketable yield (Woolhouse et al., 2005). The viruses most often reported to cause diseases in papaya include papaya apical necrosis virus (PANV), papaya ringspot virus (PRSV), papaya mosaic virus (PapMV), papaya leaf curl virus (PaLCV), tobacco ringspot virus (TRSV), papaya leaf distortion mosaic virus (PLDMV), *Papaya meleira* virus (PMeV), papaya lethal yellowing virus (PLYV), and several other viruses that may not be of economic significance (Mishra et al., 2016).

Among the diseases infecting papaya, papaya ringspot disease is the most important biotic constraint worldwide. The disease is highly destructive, threatening both small- and large-scale papaya growers in various parts of Kenya (Ombwara et al., 2014; Rimberia and Wamocho, 2014; Mumo et al., 2020). The impact of the disease is being felt in the country, with farmers in different regions of Kenya abandoning papaya cultivation in favor of other crops (Mumo et al., 2021). This calls for an urgent need to develop disease management measures.

Papaya ringspot disease in Kenya has been reportedly associated with a potyvirus MWMV (Mumo et al., 2020), as well as other viruses such as cowpea mild mottle virus (CpMMV) and papaya mottle-associated viruses (PaMV and PaMMV). The occurrences and distribution of these viruses in the country are scarcely known, although this information is crucial for disease management and prevention (Gashaw et al., 2014). The objective of this study, therefore, was to establish the incidence, prevalence, severity, and distribution of the viruses associated with papaya ringspot disease in the country.

MATERIALS AND METHODS

Sampling sites and sampling procedure

Surveys of papaya fields and the sampling of papaya plants were conducted between January and April 2017 in 22 counties. These counties include Taita Taveta, Kwale, Kilifi, Kisumu, Homabay, Migori, Siaya, Bungoma, Busia, Vihiga, Nakuru, Baringo, Elgeyo Marakwet, Kiambu, Murang'a, Kirinyaga, Embu, Tharaka Nithi, Meru, Makueni, Machakos and Kitui. Fields with papaya crops, whether established as a pure stand or intercropped, were purposefully surveyed along selected routes. In each county, a specific representative route that captured the area of interest was discussed and agreed upon by the survey team and adopted. Factors considered for the selection of routes included the sample area and the availability of suitable papaya fields. When farmers resided within the same county and papaya fields were close to each other, sampling was done at a minimum distance of 5 km between fields; otherwise, a distance interval of 10 km between fields was adopted. A transect was drawn diagonally in the field from both directions, resulting in two transects (Sseruwagi et al., 2004). During sampling, representative plants were randomly selected along X-shaped transects in each field to reduce biases. In total, 103 papaya fields were surveyed.

Incidence, severity and prevalence of papaya ringspot in selected counties in Kenya

Twenty plants per field were visually inspected for papaya ringspot symptoms on leaves, stems, petioles, and fruits. The general vigor of the inspected plants was also recorded. The disease severity scale was based on the area or proportion of symptomatic plant tissue. The scale from 1 to 5 (Ombwara et al., 2014) was adopted (Table 1). Scores of '1' (no visible symptoms) were excluded when calculating the mean severity per field to allow for a true evaluation of the degree of damage caused to the diseased plants. Disease incidence was determined as the proportion of the plants showing symptoms out of 20 examined, expressed as a percentage. The prevalence of papaya ringspot was determined in every study county as the proportion of fields with at least one diseased plant out of the total number of fields observed in that county, expressed as a percentage.

Sample collection and virus detection

Two hundred (200) symptomatic and 87 asymptomatic leaf samples were randomly collected from 2 to 5 plants per field. This involved harvesting the second youngest fully developed leaf from the shoot apex of symptomatic and asymptomatic plants using sterile forceps. The number of papaya leaf samples collected per field depended on the disease severity across the field and the plant population. The collected leaf samples were preserved in RNAlater™ (Invitrogen™) stabilization solution to prevent RNA degradation and were transported to the Biosciences Eastern and Central Africa–International Livestock Research Institute (BecA-ILRI) Hub, Nairobi laboratory, and stored at 4°C before RNA extraction.

RNA extraction and PCR process

Leaf samples were removed from the RNAlater™ solution using sterile forceps, and any remaining solution was blotted away using a sterile absorbent paper towel. Total RNA was extracted from the samples using the RNeasy® Plant Mini Kit (Qiagen, Inc.) following the manufacturer's instructions. The integrity of the extracted RNA was assessed through agarose gel electrophoresis, where 0.8% agarose was dissolved in 100 ml of 0.5X TAE (Tris-acetate-EDTA) buffer, stained with 3 µl of GelRed® Nucleic Acid Gel Stain (Biotium), and run at 100 V for 30 min in a gel tank. The gel was visualized using a gel imaging system with a UV transilluminator. The quantity of RNA was measured using the Qubit™ 2.0 Fluorometer system (Invitrogen™) following the manufacturer's instructions and normalized to 5 µg before cDNA synthesis. The cDNA was synthesized using the SuperScript™ III First-Strand Synthesis System (Invitrogen™) and stored at -20°C for use as a template in the PCR process.

Samples were screened for viruses in PCR using a set of primers specific to the respective viruses: 5' TCTCAGCTAGCACGCAACAA

E-mail: naomi.mumo@jkuat.ac.ke.

Table 1. Scale used in rating disease severity in papaya plants during field survey.

Scale	Symptoms expressed
1	No visible symptoms
2	1-25% of plant tissues portraying symptoms such as mild mottling and mild mosaic patterns on the leaves, little distortion of leaves, mild oily streaked petioles/stems, apparent but negligible stunting
3	26-50% of plant tissues portraying symptoms: moderate yellow and mosaic patterns on the leaves, moderate distortion of leaf shape, moderate oily streaked petioles/stems, moderate stunting, moderate ringspots symptoms on fruits
4	51-75% of plant tissues portraying symptoms: severe yellow and mosaic patterns on leaf, severe leaf distortion with reduced size, severe oily streaked petioles/stems severe ringspots on fruits, plant partially stunted
5	more than 75% of plant tissues portraying symptoms: very severe yellow and mosaic patterns symptoms on leaf, very severe leaf distortion and reduced size, very severe oily streaked petioles/stems, plant severely stunted and very severe ringspots symptoms on fruits.

3' and 5' CGGTGTTGAGCCAAACGAAG 3' for MWMV, 5' AGACCAAAGAGTGCTTCGGG 3' and 5' TAGGAACTCCCAGTCCCTCG 3' for PaMV, and 5' AACATGGCGACAGCTGAAGA 3' and 5' GAAGAGCGACCAGTTCCCAA 3' for CpMMV (Mumo et al., 2020). These primers were designed to amplify a 315 bp fragment for MWMV, 304 bp for PaMV, and 694 bp for CpMMV.

In brief, a 10 µl PCR reaction mixture was prepared, consisting of 5 µl of AccuPower® Taq PCR 2X Master Mix (Bioneer, Korea), 3.6 µl of nuclease-free water, 0.2 µl of 10 µM each of forward and reverse primers (Macrogen), and 1 µl of cDNA (50 ng/µl). A positive control contained a sample infected with the virus, while a negative control contained nuclease-free water, used in place of nucleic acid. The PCR reactions were run on a thermal cycler (Eppendorf Mastercycler Nexus Gradient) under the following cycling conditions: 3 min at 95°C; 30 cycles of 30 s at 95°C, 30 s at 58°C, and 1 min at 72°C; and a final extension at 72°C for 5 min for all sets of primers. The amplified PCR products, alongside O'GeneRuler ™ 1-Kb plus DNA ladder (Invitrogen™, USA), were separated on 2% (wt/vol) agarose gels, and the bands were visualized under a UV transilluminator before documentation through digital photography.

Data analysis

Data on disease incidence, prevalence and severity were analyzed by computing means among the counties surveyed. The presence of viruses was scored based on the presence or absence of the right size of the amplified fragment in the gel electrophoresis.

RESULTS

Incidence, prevalence and severity of papaya ringspot in Kenya

Papaya ringspot disease was previously reported in Kenya and in this study, the disease was observed in the majority of the counties surveyed (Table 2). It was evident that the disease has a wide occurrence with an average incidence of 21.1% reported in the counties surveyed. The highest (71.4%) disease incidence was reported in Kiambu County, followed by Murang'a and Nakuru counties with means of 51.4 and 52.8%, respectively. The least incidence was recorded in Busia County with a mean of 2.8%. However, Bungoma and Siaya counties had zero incidences (Table 2). The PRSD prevalence differed within the counties' surveyed regions with an average of 65.5%. Elgeyo Marakwet, Embu, Homabay, Kiambu, Nakuru, Kitui and Vihiga, counties recorded the highest (100%) disease prevalence, while No disease prevalence was observed in Bungoma and Siaya counties. Generally, mild disease severity (2.9) across the counties was recorded, with the highest severity of 4.0 recorded in Baringo County followed by Kirinyaga and Murang'a counties with a mean of 3.8. The least disease severities were recorded in Kwale, Kilifi and Taita Taveta counties with a mean of 2.0, while no disease severity was recorded in Bungoma and Siaya counties (Table 2).

Viruses associated with PRSD in Kenya

The viruses detected in the collected samples are shown in Table 3, and based on the detection of the respective viruses in the samples by PCR (Figure 1). A sharp band of 315, 304 and 694 bp in gel electrophoresis indicated the presence of the MWMV, PaMV and CpMMV respectively. When only one virus was detected in a sample, it is reported as a single viral detection, while in cases where more than one virus was detected in the same field surveyed and sampled, mixed infections are reported (Figure 1 and Table 3). Dual infections occurred when more than one virus was amplified in a sample.

From the PCR-based detection, 180 of 287 samples collected tested positive for at least one virus infection. MWMV was the most widespread virus detected alone, in mixed infections and dual infections. The virus was reported in 11 of 22 counties surveyed; namely, Nakuru, Busia, Homabay, Kisumu, Migori, Embu, Kiambu, Kirinyaga, Meru, Makueni, Murang'a, and Machakos, and in 140 of 287 samples collected (Table 3). PaMV was the second most widespread virus and was detected in 9 of 22 counties surveyed; namely, Baringo, Embu, Kirinyaga, Meru, Tharaka Nithi, Kitui Machakos and Taita Taveta and in 39 of 287 samples collected (Table 2). CpMMV was the least prevalent virus and was detected in only three counties including Baringo, Meru and Kitui (Table 3).

Mixed infections of MWMV and PaMV were detected in

Table 2. Incidence, prevalence and severity of papaya ringspot in major papaya-producing counties of Kenya.

County	Disease incidence (%)	Disease prevalence (%)	Disease severity
Baringo	7.7	75	4.0
Bungoma	0.0	0	1.0
Busia	2.8	50	3.0
Elgeyo Marakwet	7.2	100	2.7
Embu	35.4	100	3.7
Homabay	20.3	100	3.2
Kiambu	71.4	100	2.7
Kilifi	6.7	33.3	2.0
Kirinyaga	36.0	77.7	3.8
Kisumu	13.3	50.0	3.1
Kitui	19.4	100.0	2.9
Kwale	3.8	25.0	2.0
Machakos	33.8	90.9	3.2
Makueni	12.9	57.1	2.5
Meru	36.9	75.0	3.1
Migori	38.1	50.0	2.8
Murang'a	51.4	100	3.8
Nakuru	52.8	100	3.1
Siaya	0.0	0.00	1.0
Taita Taveta	12.4	33.3	2.0
Tharaka Nithi	11.9	45.5	2.4
Vihiga	14.3	100.0	3.3
Mean	21.1	65.5	2.9
LSD (P=0.05)	2.6		3.0

Severity was visually assessed using a scale of 1-5.
Source: Ombwara et al. (2014).

samples collected from 5 of 22 counties; namely, Embu, Kirinyaga, Meru, Machakos and Makueni while that of PaMV and CpMMV were detected in Baringo, Meru and Kitui counties. The presence of all three viruses (MWMV, CpMMV and PaMV) was obtained in Meru County (Table 3 and Figure 1D). Detections of more than one virus in fields sampled were encountered in some counties. Fifteen samples from 4 of 22 counties; namely, Embu, Kirinyaga, Machakos and Makueni had dual infections of MWMV and PaMV, while 2 of 8 samples from Kitui County were co-infected with CpMMV and PaMV (Table 3).

Detection of PRSD symptoms signified the presence of viral infection in some counties. In other instances, the presence of PRS-like symptoms was not an indicator of viral presence or absence. In Vihiga County, for instance, plants displayed symptoms and none of the three viruses was detected in them. In Baringo, Migori, Embu, Kiambu, Tharaka Nithi and Taita Taveta counties, 8/13, 10/10, 28/28, 10/10, 3/12 and 4/12 samples, respectively, displayed symptoms; however, the viruses were detected in 3/13 (1 PaMV and 2 CpMMV), 4/10 (MWMV), 17/28 (15 MWMV and 2 PaMV), 9/10 (MWMV), 2/12 (PaMV) and 3/12 (PaMV) in the respective order in each county. In some instances, the number of plants infected with viruses was higher compared to the number of symptomatic plants. For instance, in Kirinyaga, Makueni and Kisumu counties, 28/42, 13/25 and 6/12 plants respectively displayed symptoms whereas viruses were detected in 40/42 (40 MWMV and 4 MWMV+PaMV), 19/25 (8 MWMV and 11 PaMV) and 10/12 (MWMV) plants, respectively (Table 3). The most prevalent symptoms in plants included vein clearing, mosaic patterns, mottling, leaf distortion, puckering, shoe-stringing on leaves, water-soaked marks on the petioles and stems, ringspots on fruits, and stunted growth (Figure 2).

Papaya plants singly infected with MWMV displayed puckering, vein clearing, leaf distortion, shoe stringing, mottling water-soaked marks on stems and petioles, ringspots on fruits and stunted growth. On the other hand, papaya plants infected with PaMV displayed mottling, puckering and leaf distortion symptoms (Figure 2 and Table 3). The symptoms of plants in dually infected fields with MWMV and PaMV were severer, including leaf distortion, mosaic, mottling, vein clearing, ringspots, water-soaked marks, shoe stringing, puckering and stunted growth (Figure 2 and Table 3). Papaya plants infected with PaMV and CpMMV showed mild symptoms such as mottling and stunted growth.

Table 3. Incidence (%) of viruses associated with papaya ringspot in 22 counties of Kenya as determined through PCR approach.

County	No. of samples collected[a]	No. of symptomatic samples[b]	Symptoms [c]	MWMV	PaMV	CpMMV	MWMV+PaMV	CpMMV +PaMV
Baringo	13	8	Mo	-	1	2	-	-
Elgeyo marakwet	8	2	SG, M	-	-	-	-	-
Nakuru	7	7	Mo, LD, RS, SG	7	-	-	-	-
Bungoma	3	0	None	-	-	-	-	-
Busia	4	2	WS	2	-	-	-	-
Homabay	14	14	PU, VC, WS, LD, SG	14	-	-	-	-
Kisumu	12	6	Mo, PU, WS, LD	10	-	-	-	-
Migori	10	10	Mo, M, VC	4	-	-	-	-
Siaya	2	0	None	-	-	-	-	-
Vihiga	2	2	Mo, VC	-	-	-	-	-
Kiambu	10	10	Mo, RS, WS, VC, LD, SG	9	-	-	-	-
Kirinyaga	42	28	LD, Mo, VC, RS, WS, SS, PU, SG	40	4	-	4	-
Meru	13	10	VC, M, Mo, PU, LD, SS, SG, WS	4	4	2	2	-
Murang'a	16	12	LD, M, RS, Mo, VC, SS, WS, PU	12	-	-	-	-
Tharaka Nithi	12	3	Mo	-	2	-	-	-
Embu	28	28	LD, VC, PU, M, Mo, WS, LC	15	2	-	2	-
Kitui	8	8	Mo, SG	-	5	3	-	2
Machakos	26	22	Mo, PU, RS, SS, WS, SG	15	7	-	5	-
Makueni	25	13	Mo, LD, WS, M, PU, RS, SG	8	11	-	2	-
Kwale	12	6	Mo, M	-	-	-	-	-
Kilifi	8	2	Mo, LD	-	-	-	-	-
Taita taveta	12	4	Mo, PU, LD	1	2	-	-	-
Total	**287**	**200**		**140**	**39**	**7**	**15**	**2**

[a]Number of samples collected per county for virus detection using PCR approach. [b]Number of samples collected from plants exhibiting papaya ringspot symptoms. [c]symptoms exhibited by plants M: Mosaic patterns on the leaves; Mo: Mottling symptoms on the leaves; VC: vein clearing; PU: Puckering; SS: shoe stringing; LD: Leaf distortion: WS: Water-soaked marks on stems and petioles; RS: Ringspots on fruits and; SG: Stunted growth of the plant. (-), not detected; MWMV, Moroccan watermelon mosaic virus; PaMV, Papaya mottle virus; CpMMV, Cowpea mild mottle virus.

DISCUSSION

Papaya ringspot disease is a major threat to papaya production in Kenya. The impact of the disease in the country is becoming serious that many growers have abandoned the fruit crop in favour of other crops (Mumo et al., 2021). This study provides information on the incidence, severity and prevalence of PRSD and maps out its distribution which is important aspects for the development of an effective management approach.

Papaya plants showing symptoms associated with the disease were observed in 20 out of 22 counties surveyed, causing minimal to severe levels of damage. Prevalence levels of up to 100% were also reported in some counties signifying the widespread and threat of the disease to papaya production in the country. The highest disease severities were reported in Kirinyaga, Murang'a Makueni, Machakos and Kiambu counties.

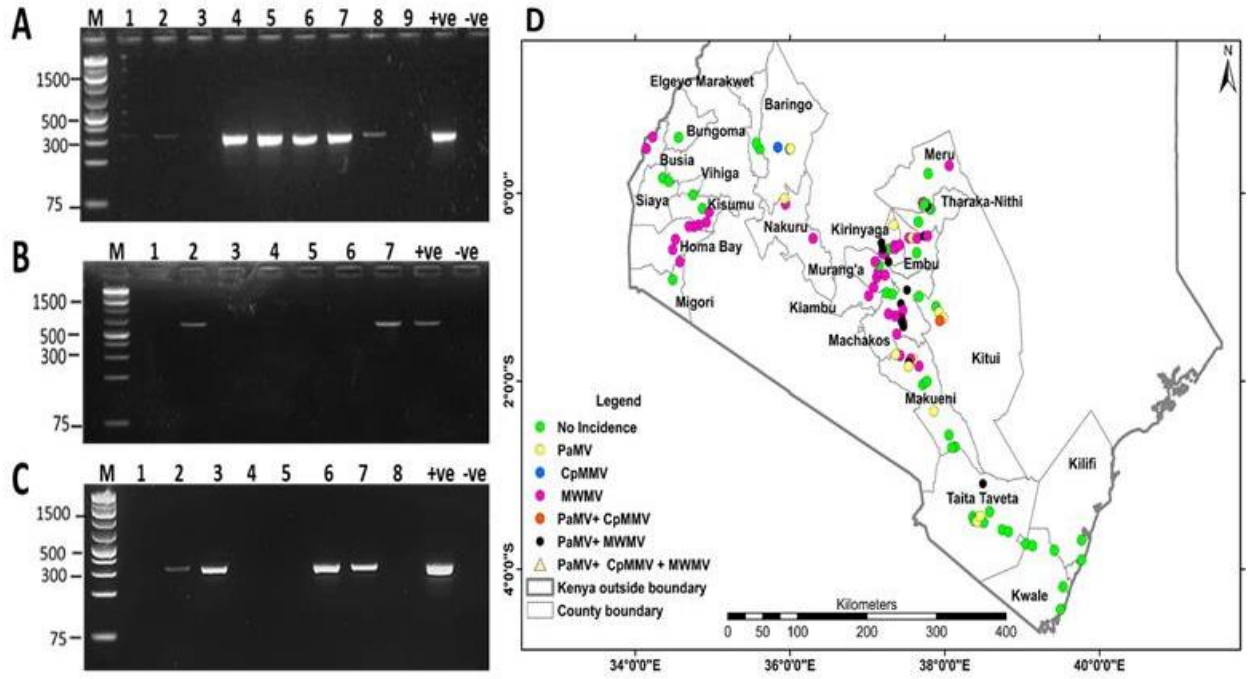

Figure 1. Gel electrophoresis for diagnostic studies of MWMV PaMV and CpMMV in Kenyan papaya. A band at 315 bp in (A), 694 bp in (B) and 304 bp in (C) show the presence of MWMV, CpMMV and PaMV, respectively. M indicates the O'GeneRuler™ 1 kb plus DNA ladder. +ve is a positive control, -ve is negative control. Numbers 1-9 = papaya samples. (D) A section of the map of Kenya showing combinations of viruses associated with the disease as determined through RT-PCR approach in selected counties in Kenya. The map was developed using QGIS software.
Source: QGIS Development Team (2019).

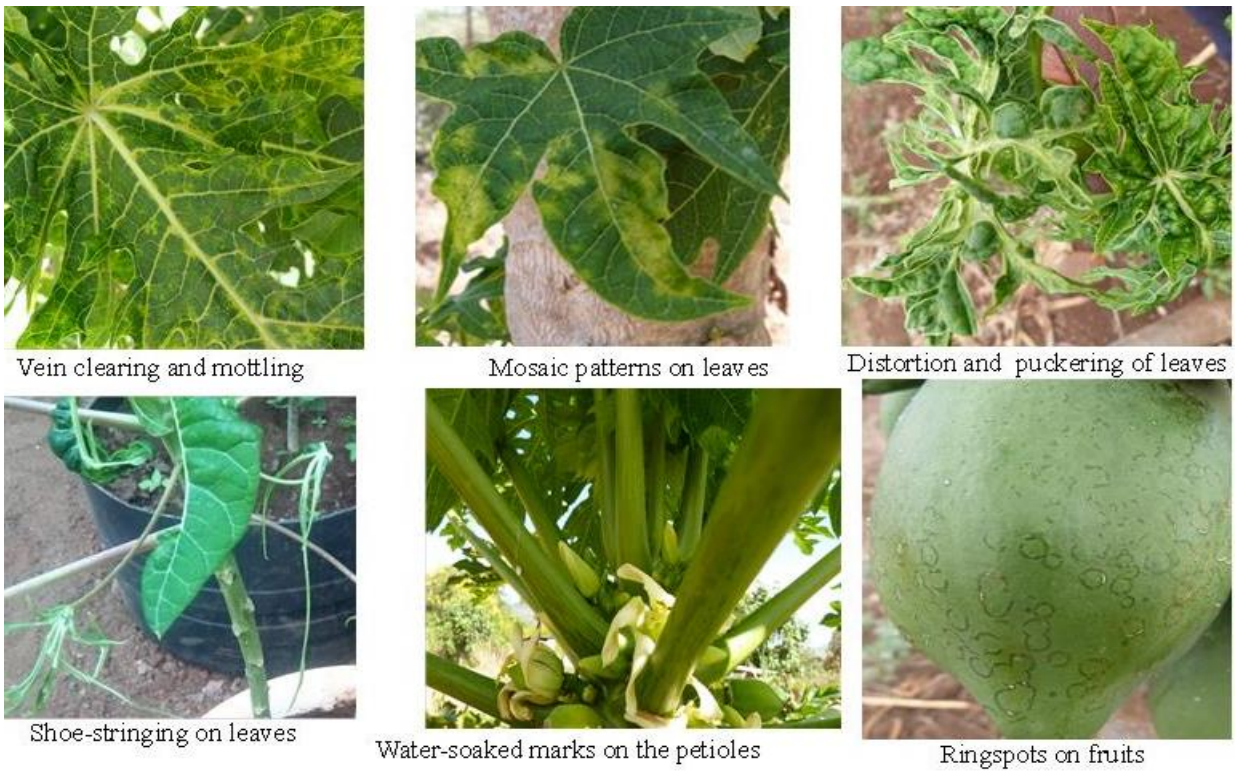

Figure 2. Symptoms displayed by papaya plants infected by viruses associated with the disease.

The situation could partly be attributed to a lack of management measures as observed during the survey due to minimal knowledge of the disease and its causal agents (Mumo et al., 2021). In these counties, some farmers also cultivated papaya as a mono-crop on large fields for commercial purposes, which could have encouraged fast disease spread because of the high host density and large size of cropped area (Piper et al., 1996; Kumar et al., 2010). Furthermore, monoculture facilitates easy movement of vectors from plant to plant during their transitional flights as they probe for a suitable host (Kumar et al., 2010), a situation that could contribute to the high disease incidences in these counties.

Three viruses, MWMV, PaMV and CpMMV were detected in both symptomatic and asymptomatic papaya samples collected during the survey in farmers' fields in the major growing counties in Kenya. MWMV was the commonest and was widely distributed. The virus is one of the most common cucurbit viruses in Africa (Lecoq et al., 2001; Yakoubi et al., 2008; Ibaba et al, 2016; Kidanemariam et al., 2019). Although the virus was reported for the first time in papaya more than a decade ago in Congo (Arocha et al., 2008), its wide distribution in the country indicates that the virus is well established in papaya and there is an urgent need to develop management strategies is of paramount importance. The PaMV was recently discovered and described as a 'new' virus infecting papaya in Kenya (Mumo et al., 2020). However, little is known about its impact on papaya crops, its vectors and mode of transmission as well as alternate hosts. Nevertheless, the virus poses a serious production challenge to papaya because of its wide distribution and occurrences of dual infections with other viruses. The CpMMV infecting papaya is recombinant (Mumo et al., 2020) and its incidences in papaya production counties are very low. The detection and low incidences of CpMMV in papaya could be attributed to the recent host jump from cowpea to papaya after recombination and mutation leading to an increase in the host range (Legg and Thresh, 2000; Monci et al., 2002; Woolhouse et al., 2005). The CpMMV has been reported in leguminous and solanaceous crops in Africa (Jeyanandarajah and Brunt, 1993). During the survey, it was observed that cowpea plants were intercropped with papaya. Therefore, there is a chance that the whitefly transmitted the virus from cowpea to papaya, but this needs to be confirmed empirically.

Some plants displayed papaya ringspot symptoms (Table 3), although no viruses were detected. For example, in Baringo, Migori, Embu, Kiambu, Tharaka Nithi and Taita Taveta counties, the number of plants infected was lower compared to the number of symptomatic plants. The absence of viruses in symptomatic plants could be attributed to other viral or non-viral diseases, nutrient disorders, insect damage (Schreinemachers et al., 2015) and viral load/titer and or existence of variants which may not be detected by the primers used (Ghoshal and Sanfacon, 2015). In other instances, the number of plants infected with the viruses was higher compared to the number of symptomatic plants (for example in Kirinyaga, Makueni and Kisumu counties). The absence of symptoms on virus-infected plants could probably be because the plants had just been infected and had not developed symptoms at the time the survey was carried out, the time of the year/season when the plant was infected, antagonisms due to co-infection with another virus or tolerance of the plant to the viruses (Mowlick et al., 2008; Kumar et al., 2010; Singh and Shukla, 2011).

The distribution of individual virus infections in Kenya is not region-specific. For instance, single PaMV infections occurred in Tharaka Nithi (Eastern) and Taita Taveta (Coast); while MWMV single infections were recorded in Kiambu, Murang'a, Nakuru, Kisumu, Homabay, Migori and Busia counties, which are either located in Central, Rift Valley or Western. The difference may be a result of the different frequencies of distribution of individual viruses. The two viruses, PaMV and MWMV were found in Kirinyaga, Embu, Makueni and Machakos Counties, which are located in the central and eastern regions. The PaMV and CpMMV were found in Baringo (Rift valley), Meru (Central) and Kitui (Eastern) Counties. No dual infection with MWMV and CpMMV was detected.

CONCLUSION AND RECOMMENDATIONS

This study has successfully determined the incidence, severity, prevalence, and distribution of papaya ringspot-associated viruses in Kenya. These viruses are prevalent across various counties and may potentially be spreading to unreported areas. Co-infections of these viruses have also been observed. Papaya ringspot exhibits peculiar patterns of prevalence and symptom development, influenced by varying weather conditions. In some instances, symptoms may be masked in infected plants, depending on the seasons (Stevens, 1983; Mowlick et al., 2008).

As a result, it is crucial to implement continuous monitoring and surveillance of these viruses to assess potential variations in symptoms and prevalence throughout the year. Simultaneously, management measures should be enforced to curb the spread of the disease. These measures include the use of virus-free planting materials, roguing of infected plants, restrictions on the movement of seedlings from one region to another, and certification for the production of clean seedlings in regions not yet infested.

Furthermore, it is essential to investigate the effects of co-infections of these viruses on papaya plants. Additionally, we should explore the possibility of other viruses causing symptoms in samples where no viruses were initially detected. While this study, conducted in 2017, has provided a valuable baseline for understanding the incidence of viral diseases, further studies conducted

over time will be necessary to establish the long-term patterns of distribution and severity of these diseases in papaya cultivation in Kenya.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Arocha Y, Vigheri N, Nkoy-Florent B, Bakwanamaha K, Bolomphety B, Kasongo M, Betts P, Monger WA, Harju V, Mumford RA, Jones P (2008). First report of the identification of Moroccan watermelon mosaic virus in papaya in Democratic Republic of Congo. Plant Pathology 57(2):387.

Gashaw G, Alemu T, Tesfaye K (2014). Evaluation of disease incidence and severity and yield loss of finger millet varieties and mycelial growth inhibition of Pyricularia grisea isolates using biological antagonists and fungicides in vitro condition. Journal of Applied Biosciences 73:5883-5901.

Ghoshal B, Sanfaçon H (2015). Symptom recovery in virus-infected plants: Revisiting the role of RNA silencing mechanisms. Virology 479:167-179.

HCDA (2021). Horticulture Validated report. Nairobi, Kenya. Nairobi, Kenya. Retrieved from www.agricultureauthority.go.ke/.../Horticulture-Validated-Report-2014-Final-copy.pd

Ibaba JD, Laing MD, Gubba A (2016). Genome sequence analysis of two South African isolates of Moroccan watermelon mosaic virus infecting cucurbits. Virus Genes 52(6):896-899.

Jeyanandarajah P, Brunt AA (1993). The Natural Occurrence, Transmission, Properties and Possible Affinities of Cowpea Mild Mottle Virus. Journal of Phytopathology 137(2):148-156.

Kansiime MK, Rwomushana I, Mugambi I, Makale F, Lamontagne-Godwin J, Chacha D, Kibwage P, Oluyali J, Day R (2020). Crop losses and economic impact associated with papaya mealybug (Paracoccus marginatus) infestation in Kenya. International Journal of Pest Management 0(0):1-14.

Kidanemariam DB, Sukal AC, Abraham AD, Njuguna JN, Stomeo F, Dale JL, James, Harding MR, James AP (2019). Molecular characterisation of a putative new polerovirus infecting pumpkin (Cucurbita pepo) in Kenya. Archives of Virology 1(0123456789):0-4.

Kumar NKK, Singh HS, Kalleshwaraswamy CM (2010). Aphid (aphididae: Homoptera) vectors of papaya ringspot virus (PRSV), bionomics, transmission efficiency and factors contributing to epidemiology. Acta Horticulturae 851:431-442.

Lecoq H, Végétale SDP, Cedex M, Dafalla G, Medani W, Desbiez C, Wipf-scheibel C (2001). Biological and Molecular Characterization of Moroccan watermelon mosaic virus and a Potyvirus Isolate from Eastern Sudan. Plant Disease 85(5):547-552.

Legg JP, Thresh JM (2000). Cassava mosaic virus disease in East Africa: A dynamic disease in a changing environment. Virus Research 71(1-2):135-149.

Mishra R, Kumar RG, Patil BL (2016). Current Knowledge of Viruses Infecting Papaya and Their Transgenic Management. In R. K. G. et Al. (Ed.), Plant Viruses: Evolution and Management. New Delhi 110012, India: © Springer Science+Business Media Singapore pp. 189-204.

Monci F, Sánchez-Campos S, Navas-Castillo J, Moriones E (2002). A natural recombinant between the geminiviruses Tomato yellow leaf curl Sardinia virus and Tomato yellow leaf curl virus exhibits a novel pathogenic phenotype and is becoming prevalent in Spanish populations. Virology 303(2):317-326.

Mowlick S, Akther MS, Kundu BC, Akanda AM (2008). Masking behaviour and quantitative assessment of growth and and yield reduction of papaya due to papaya ringspot virus. Bangladesh Research Publications Journal 1(3):206-214.

Mumo NN, Mamati GE, Ateka EM, Rimberia FK, Asudi GO, Boykin LM, Machuka EM, Njuguna JN, Pelle R, Francesca S (2020). Metagenomic Analysis of Plant Viruses Associated With Papaya Ringspot Disease in *Carica papaya* L. in Kenya. Frontiers in Microbiology, 11. https://doi.org/10.3389/fmicb.2020.00205

Mumo NN, Mamati GE, Ateka EM, Rimberia FK, Asudi GO (2021). Farmers' Knowledge, Perception and Management Practices af Papaya Ringspot Disease in Kenya. African Journal of Horticultural Science 18(2):31-42.

Ombwara FK, Asudi GO, Rimberia FK, Ateka EM, Wamocho LS (2014). The Distribution and Prevalence of Papaya Ring Spot Virus (PRSV) in Kenyan Papaya. Acta Horticulturae (1022):119-124.

Piper JK, Handley MK, Kulakow PA (1996). Incidence and severity of viral disease symptoms on eastern gamagrass within monoculture and polycultures. Agriculture Ecosystems and Environment 59:139-147.

QGIS Development Team (2019). QGIS Geographic Information System; Open Source Geospatial Foundation Project, 2019. https://qgis.org/

Rimberia FK, Wamocho LS (2014). Papaya industry in Kenya: Production, consumption and outlook. Acta Horticulturae (1022):181-188.

Schreinemachers P, Balasubramaniam S, Boopathi NM, Ha CV, Kenyon L, Praneetvatakul S, Sirijinda A, Le NT, Srinivasan R, Wu MH (2015). Farmers' perceptions and management of plant viruses in vegetables and legumes in tropical and subtropical Asia. Crop Protection 75:115-123.

Singh V, Shukla K (2011). Influence of inoculation time on severity of virus disease caused by Papaya ringspot virus. Annals of Plant Protection Sciences 19(1):142-146.

Sseruwagi P, Sserubombwe WS, Legg JP, Ndunguru J, Thresh JM (2004). Methods of surveying the incidence and severity of cassava mosaic disease and whitefly vector populations on cassava in Africa: A review. Virus Research 100(1):129-142.

Stevens WA (1983). Virology of flowering plants (1st ed.). Springer, Boston, MA. https://doi.org/110.1007/978-1-4757-1251-3

Woolhouse MEJ, Haydon DT, Antia R (2005). Emerging pathogens: The epidemiology and evolution of species jumps. Trends in Ecology and Evolution 20(5):238-244.

Yakoubi S, Desbiez C, Fakhfakh H, Wipf-Scheibel C, Marrakchi M, Lecoq H (2008). Biological characterization and complete nucleotide sequence of a Tunisian isolate of Moroccan watermelon mosaic virus. Archives of Virology 153(1):117-125.

Related Journals: