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EFFECTS OF SINGLE AND MIXED VIRUS INFECTIONS ON THE PERCENT GERMINATION OF SOME COWPEA (*Vigna unguiculata* [L.] Walp) CULTIVARS

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SUMMARY

A field trial was carried out to assess the response of twenty-five cultivars of cowpea to single and mixed infections with *Blackeye cowpea mosaic virus* (BlCMV) and *Cowpea mottle virus* (CPMoV) on seed quality. The field trial was conducted at the Teaching and Research Farm of the Faculty of Agriculture, Ahmadu Bello University (ABU), Zaria, Mokwa Station ($09^0211'N$ and $5^0135'E$, 201 m above sea level) situated in the Southern Guinea Savannah agro - ecological zone of Nigeria. The seed viability test was determined at the Crop Production Laboratory, Department of Crop Production, Federal University of Technology, Minna, Nigeria. Four independent trials were conducted simultaneously, for single and mixed infections. The field was cleared, ploughed, harrowed and ridged at 0.75 m apart then marked out into plots and replications. The trial was a randomized complete block design (RCBD) replicated three times giving a total land area of 900 m^2. Three cowpea seeds of each cultivar were sown after dressing with Apron – star (methylthiuram + metalaxyl + carboxin) at the of rate 3.0 kg seed per 10 g of the chemical. Seeds were sown at an intra and inter– row spacing of 0.30 × 0.75 m along the ridges and later thinned to two per stand at 2 weeks after sowing (WAS). For the single virus infection, seedlings of the twenty five cultivars were inoculated at 10 days after sowing (DAS) while for the mixed virus infections,

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seedlings were inoculated at 10 and 17 DAS. The results of the experiment revealed that all cultivars were susceptible to single and mixed infections of the two viruses but to seemingly different extents. The viability of seeds from single infection with CPMoV was slightly reduced in some instances, but, even when seeds viability was not much affected, test of accelerated ageing for four weeks indicated that seed vigour was seriously impaired as compared to the other three virus treatments.

Keywords: *Blackeye cowpea mosaic virus*, *Cowpea mottle virus*, cowpea seeds, Seed quality, Germination

COWPEA (*Vigna unguiculata* [L.] Walp) is one of the ancient crops known to man. Its origin and domestication occurred in Africa near Ethiopia and subsequently was developed mainly in the farms of the African Savannah (12). Today, it is widely adapted and grown throughout the world but Africa predominates in production. It is a major staple food crop in sub-Saharan Africa, especially in the dry savanna regions of West Africa (9). The seeds are a major source of plant protein and vitamins for man, feed for animals, and also a source of cash income. The young leaves and immature pods are eaten as vegetables (9). It has been estimated that the annual world cowpea crop is grown on 12.5 million hectares, and the total grain production is 3.9 million tonnes (11). More than 8 million hectares of cowpea are grown in West and Central Africa. Also, it is known that Nigeria is the largest producer with 4 million hectares accounts for 45 % of the total on 1.15 million hectares annually (9). Other producers are Niger, Mali, Burkina Faso and Senegal (12). The major cowpea producing areas in Nigeria include Niger, Kwara, Kaduna, Borno, Taraba and Yobe States in the northern part while Oyo, Ogun and Ondo also produce appreciable quantities in the southern part of the country (14).

Virus diseases are considered to be a major limiting factor for the production and productivity of legumes in the tropical and sub-tropical countries (6). Out of more than 20 viruses reported on legumes from different parts of the world, (15) nine are known to infect cowpea naturally in Nigeria. *Blackeye cowpea mosaic virus* was first reported on cowpea in the U.S. in 1955 (4). It is distributed in all ecological zones and cowpea-

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growing areas of Nigeria. Local symptoms appear as large reddish lesions that spread along the veins, while systemic symptoms appear as severe mottle, mosaic, vein-banding, veinal chlorosis, distortion and stunting of the plant. Disease symptoms vary with virus strain and host cultivar. Incidence varies from 1-40 % on farmers' fields. Yield losses due to the virus vary from 10-85 % on individually infected plants and vary with time of sowing.*Cowpea mottle virus*is a positive sense single-stranded RNA, unipartite, isometric virus, 30 nm in diameter (4). The pathogen is distributed in all ecological zones of Nigeria, particularly in the riverine areas of the middle belt which has a Southern Guinea Savanna climate and where a lot of bambara groundnut is grown. Infected plants display severe mosaic, mottling or bright yellow mosaic. Leaf distortion and reduction in leaf size sometimes leading to a witches' broom appearance in cowpea occurs (8).

Seed-borne viruses are important for source of diseases at the beginning of production even at low rates of seed transmission (15). In addition, seed-borne viruses can aggravate other transmission methods and cause disease to spread rapidly. Seed-borne and seed transmitted viruses are also damaging to cowpea productivity owing to inherent primary inoculum and potential for their wide dispersal. Information on the possibility of seed transmission in virus infected cowpeas will be valuable to numerous cowpea farmers. Information on germination of infected seeds and survival of resulting plants, virus disease progress during the growing season, magnitude of yield loss and amount of infection in harvested seeds in replicated field experiments is required to establish acceptable threshold levels of seed-borne infections. The study is essential to develop preventive and management measures for cowpea virus diseases in Niger State. Therefore, this research aimed at examining the effects of virus infections on seed quality.

MATERIALS AND METHODS

Field trial was conducted during the 2017 wet session at the Teaching and Research farm of the Faculty of Agriculture, Ahmadu Bello University (ABU), Mokwa Station ($09^0211'$N and $5^0135'$E, 201 m above sea level) situated in the Southern Guinea Savannah agro - ecological zone of Nigeria. The site used was under continuous cropping with soybean between 2012 till the commencement of the study.

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Treatments and experimental design

Four independent trials were conducted simultaneously, for single and mixed infections of the two most common viruses in the study area. In each trial, 25 photosensitive and yielding cowpea cultivars under virus free conditions namely Ife Brown, IT90K – 277 – 2, IT96D – 610, IT97K – 499 – 35, IT97K – 568 – 18, IT97K – 573 – 2 –1, IT98K – 205 –M8, IT98KD – 288, IT99K – 316 – 2, IT99K – 377 – 1, IT00K – 901 – 5, IT03K – 337 – 6, IT04K – 267 – 8, IT04K – 291 – 2, IT04K – 321 – 2, IT04K – 332 – 1, IT06K – 124, IT06K – 137 – 1, IT07K – 211 – 1 – 8, IT07K – 222 – 2, IT07K – 243 – 1 – 10, IT07K – 251 – 3 – 3, IT07K – 292 – 1 – 10, IT07K – 299 – 6 and IT07K – 318 – 33) constituted the treatments. The trial was arranged as randomized complete block design (RCBD) replicated three times giving a total land area of 900 m^2.

Source of inoculum and multiplication

The *Blackeye cowpea mosaic virus* (BICMV) and *Cowpea mottle virus* (CPMoV) isolates used were obtained from the Department of Crop Production, Federal University of Technology, Minna Niger State. The isolates were identified through serological test at the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The virus isolates were extracted by grinding 1g/ml of each isolate in extraction buffer containing 0.1M sodium phosphate dibasic, 0.1M potassium phosphate monobasic, 0.0lM ethylene diamine tetra acetic acid and 0.00lM-cystine per litre of distilled water using a pre-cooled sterilized mortar and pestle as described by Kumar (16). Two microlitres of β- mercapto-ethanol was added to the extract just before use. Cowpea seedlings were infected with BICMV and CPMoV inoculum at under sreenhouse 10 days after sowing (DAS) by rubbing the virus extracts on the upper surface of the leaves that was dusted with carborundum powder (600- mesh). The leaves of inoculated plant were rinsed with sterile distilled water. Symptomatic cowpea leaves were collected from the infected plants at 3 weeks after inoculation (WAI) and used for inoculation during the main experiment. The leaves were preserved at room temperature in airtight via bottle on silica gels covered with a thin layer of non-absorbent cotton wool.

Agronomic practices

The field was cleared, ploughed, harrowed and ridged with tractor at

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0.75 m apart then marked out into plots and replications. Each cultivar was evaluated in 0.375 m ridge wide, 3 m long and 0.75 m apart giving a total plot size of 18.75 m per replicate. Cowpea seeds were sown one week after the land preparation. Three cowpea seeds of each cultivar were sown after dressing with Apron – star (methylthiuram + metalaxyl + carboxin) at the of rate 3.0 kg seed per 10 g sachet of the chemical to protect seed against soil borne pathogens. The sowing was carried out at an intra and inter– row spacing of 0.30 × 0.75 m along the ridges and later thinned to two per stand at 2 weeks after sowing (WAS). The BICMV and CPMoV infected cowpea leaves previously preserved on silica gels were used for inoculation. For the single virus infection, seedlings of the twenty five cultivars were mechanically inoculated singly with BICMV or CPMoV at 10 days after sowing while for the mixed virus infections, seedlings were inoculated singly at 10 DAS and inoculated with the second virus at 17 DAS. Weeds were manually controlled through hand weeding at 4 and 6 weeks after sowing. Insect pests were controlled by spraying D-D force (Cypermethrin plus Dimethoate) at flower initiation and pods setting. Pods were harvested at physiological maturity. The pods were processed and packaged for seed quality assessment in the laboratory.

Assessment of Virus Infection on Seed Quality

Seed lots from the various virus treatments were subjected to seed quality test as follows;

Germination and longevity of seeds of all the virus treatment combinations were determined by germination test after harvest and at four weeks of storage respectively at the Crop Production Laboratory, Department of Crop Production, Federal University of Technology, Minna. There were 25 seeds placed in distilled-water moistened filter paper lined in Petri-dish in three replicates. The filter papers in the petri-dishes were kept moist every other day. The petri-dishes were arranged inside the seed germination chamber. Germination counts were taken at 1, 2, 3, 4 and 5 days after sowing. Seeds were considered germinated when the tip of the radicle had grown free from the seed coat (10). Germination percentage (GPCT) was calculated as follows:

$$GPCT = \frac{\text{Total number of seedlings that emerged on the final day}}{\text{Total number of seeds planted}} \times 100$$

Cowpea seeds were also subjected to accelerated ageing tests at two and

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four weeks as described by El Balla *et al.* (10) for vigour determination. The seeds of all the treatments were stored in open plastic plates and arranged inside an incubator at 35 °C and 86 % relative humidity. This was aimed at accelerating the ageing of the seeds so that the relative longevity of the seed samples could be determined. Twenty-five seeds from each treatment that were artificially aged in three replications were counted and placed on layer of distilled water moistened-filter paper placed in Petri-dishes over a wire mesh screen inside a growth chamber at 30 °C in the dark. Germination count was taken as described above.

Data analysis

Data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS, 2008) to verify if there were significant differences among the cultivars. Significance was determined at 5 % level of probability. Where the *F*-test ratio was significant, means were separated using Student-Newman-Keuls (SNK) test.

RESULTS

Effects of single and mixed virus infections on cowpea seed quality

The study revealed significant impairments in germination before and after four weeks of storage of the 25 cultivars of cowpea both in single and mixed infections of the viruses used. The variation in seed germination of cowpea cultivars with respect to virus infections is presented in Table 1. Prior to storage of seeds, the difference between the lowest and highest mean value for seed germination was wide and significant ($p<0.05$). Seed germination percentage varied from 77.4 to 99.7 % for the BICMV infected cultivars, 77.4 to 98.7 % for CPMoV infected cultivars, 74.8 to 98.5 % for BICMV + CPMoV infected cultivars and 78.6 to 98.5 % for CPMoV + BICMV inoculated cultivars (Table 1).Seeds obtained from IT97K-568-18, IT04K-332-1 and IT07K-292-1-10 cowpea cultivars infected with BICMV had significantly ($p<0.05$) higher germination percentage of 99.7 which was statistically similar to 97.6 and 97.3 % germination obtained from seeds of cultivar IT07K-243-1-10 and IT03K-337-6 respectively. Seeds from cultivars IT90K-277-2, IT07K-211-1-8 and IT06K-124 had germination values of 94.7, 94.3 and 93.7 % respectively which were not significantly different among each other. Seeds of cultivars IT07K-251-3-3 and IT07K-222-2 had 92.3 and 92.5 % germination values respectively

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which were statistically similar while seeds from the remaining cowpea cultivars had germination percentages ranging between 77.4 and 91.3.

Furthermore, seed germinability of 98.7 % was highest in IT90K-277-2 with CPMoV infected cowpea seeds which was not significantly ($p > 0.05$) different from seeds obtained from cultivars IT04K-332-1 (98.5 %), IT07K-243-1-10 (98.4 %), IT04K-267-8 (98.2 %) and IT96D-610 (97.7 %), while significantly lowest seed germination percentage of 77.4 was recorded in seeds of cowpea cultivar IT07K-292-1-10 (Table 1). On the other hand, co-infections of cowpea seeds significantly ($p<0.05$) affected seed germinability across the cowpea cultivars investigated. BICMV + CPMoV infected IT04K-332-1 exhibited the highest germination percentage of 98.5 % than all other cultivars, whereas IT96D-610 and IT97K-499-35 gave 97.6 % each. Seeds of cultivars IT07K-292-1-10 and IT97K-573-2-1 had 96.0 and 94.8 % germination respectively, while seeds of cultivar IT07K-222-2 gave in the lowest germination percentage of 74.8. Seeds obtained from cultivar IT97K-568-18 infected with CPMoV + BICMV exhibited the highest germination percentage of 98.5 before storage which was not significantly ($p > 0.05$) different from 97.3 % obtained from seeds of IT99K-316-2. Next to these with high germination percentage of 96 were seeds obtained from IT90K-277-2, IT96D-610, IT98K-205-M8, IT98KD-288, IT04K-332-1 and IT07K-222-2 whereas the significantly lowest germination percentage of 78.6 was recorded in seeds of cowpea cultivars IT04K-321-2 and IT07K-211-1-8. (Table 1).

Similarly, the difference between the lowest and highest percentage mean values for the longevity test was also wide and significant ($p<0.05$) when seeds were stored for four weeks.

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Table 1: Cowpea seed quality as affected by single and mixed infections of *Blackeye cowpea mosaic virus* (BICMV) and *Cowpea mottle virus* (CPMoV) at Mokwa in 2017

	Germination Test (%)				Accelerated Ageing Germination (%) 4 Weeks of Storage			
Cultivar	BICMV	CPMoV	BI + CP	CP + BI	BICMV	CPMoV	BI + CP	CP + BI
Ife Brown	93.5^{bcd}	90.5^{c-f}	86.7^{f}	86.5^{gh}	77.9^{a}	69.0^{b}	56.6^{b}	46.2^{l}
IT90K – 277 – 2	94.7^{bc}	98.7^{a}	78.5^{c}	96.0^{bc}	76.6^{a}	68.0^{bc}	58.4^{a}	51.6^{e}
IT96D – 610	87.3^{g}	97.7^{a}	97.6^{b}	96.0^{bc}	70.3^{b}	66.5^{d}	52.0^{e}	52.0^{de}
IT97K – 499 – 35	88.0^{fg}	86.9^{i}	97.6^{b}	92.0^{e}	61.5^{de}	60.0^{g}	55.0^{c}	56.0^{ab}
IT97K – 568 – 18	99.7^{a}	91.2^{c}	81.3^{j}	98.5^{a}	69.5^{b}	57.2^{h}	48.0^{h}	41.2^{l}
IT97K – 573 – 2 – 1	87.8^{g}	93.4^{b}	94.8^{d}	94.5^{d}	50.6^{l}	57.1^{hi}	45.5^{i}	35.6^{m}
IT98K – 205 – M8	87.6^{g}	89.2^{efg}	77.5^{m}	96.0^{bc}	57.5^{gh}	70.6^{a}	56.3^{b}	41.5^{l}
IT98KD – 288	91.3^{c-g}	90.7^{cde}	82.6^{i}	96.0^{bc}	48.0^{m}	62.7^{f}	48.3^{gh}	51.3^{ef}
IT99K – 316 – 2	92.1^{c-f}	93.4^{b}	85.0^{gh}	97.3^{ab}	53.3^{jk}	64.0e^{f}	57.3^{ab}	46.0^{j}
IT99K – 377 – 1	88.9^{efg}	90.8^{cd}	85.4^{gh}	92.0^{e}	60.0ef	60.0^{g}	50.6^{f}	31.6^{n}
IT00K – 901 – 5	88.8^{efg}	86.1^{i}	81.3^{j}	89.3^{f}	70.6^{b}	65.0^{e}	52.0^{e}	47.0^{ij}
IT03K – 337 – 6	97.3^{ab}	89.4^{d-g}	84.6^{h}	89.3^{f}	50.5^{l}	66.8^{cd}	46.4^{i}	41.4^{l}
IT04K – 267 – 8	92.2^{c-f}	98.2^{a}	81.3^{j}	86.5^{gh}	56.0^{hi}	62.6^{f}	49.5^{fg}	54.6^{bc}
IT04K – 291 – 2	87.8^{g}	86.9^{i}	89.3^{e}	87.7^{g}	54.6^{ij}	58.7^{g}	57.4^{ab}	52.0^{de}
IT04K – 321 – 2	90.5^{c-g}	93.8^{b}	85.3^{gh}	78.6^{k}	58.6^{fg}	56.3^{hi}	48.0^{h}	50.6^{efg}
IT04K – 332 –	99.7^{a}	98.5^{a}	98.5^{a}	96.0^{bc}	60.0^{ef}	53.4^{k}	49.3^{fg}	48.1^{hi}

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IT06K – 124	93.7^{bc}	90.1^{c-g}	80.0^{k}	81.2^{j}	46.6^{m}	56.8^{hi}	58.6^{a}	49.6^{fg} h
IT06K – 137 – 1	77.4^{h}	87.2^{hi}	78.8^{l}	80.0^{jk}	52.0^{kl}	56.0^{hij}	54.5^{cd} e	44.0^{k}
IT07K – 211 – 1 – 8	94.5^{bc}	88.5^{gh}	89.3^{e}	78.6^{k}	53.3^{jk}	56.0^{hij}	53.4^{d}	49.3^{gh}
IT07K – 222 – 2	92.5^{cde}	93.0^{b}	74.8^{n}	96.0^{bc}	54.2^{j}	56.4^{jk}	45.3^{ij}	53.7^{bc}
IT07K – 243 – 1 – 10	97.6^{ab}	98.4^{a}	89.3^{e}	94.8^{cd}	57.5^{gh}	54.7^{jk}	50.5^{f}	50.8^{efg}
IT07K – 251 – 3 – 3	92.3^{cde}	88.5^{gh}	82.8^{i}	85.3^{h}	57.3^{gh}	56.6^{ij}	44.0^{k}	47.0^{ij}
IT07K – 292 – 1 – 10	99.7^{a}	77.4^{j}	96.0^{c}	81.3^{j}	62.1^{d}	57.3^{h}	58.3^{a}	57.3^{a}
IT07K – 299 – 6	80.3^{h}	89.0^{gh}	86.8^{f}	82.6^{i}	64.4^{c}	64.0^{ef}	49.7^{f}	44.0^{k}
IT07K – 318 – 33	89.3^{d-g}	77.6^{j}	85.6^{g}	80.0^{jk}	59.8^{f}	58.7^{g}	44.9^{jk}	46.5^{ij}
SE ±	1.27	0.5	0.26	0.43	0.54	0.46	0.42	0.61

Means with the letter (s) within the same column are not significantly ($p \leq 0.05$) different by Student-Newman-Keuls (SNK) test

Significantly highest germination percentage of 77.9 was recorded in seeds of BICMV infected Ife Brown followed by IT90K-277-2, IT00K-901-5 and IT96D-610 with 76.6, 70.6 and 70.3 germination percentage, respectively. Seeds of cultivar IT97K-568-18, IT07K-292-1-10 and IT07K-299-6 exhibited germination values of 69.5, 64.4 and 62.1 %, respectively whereas the least germination values of 46.6 % was obtained from seeds of IT06K-124. Mean value for accelerated ageing germination (AAG) on CPMoV infected cowpea cultivars showed that seeds of IT98K-205-M8 had 70.6 % germination. This was closely followed by seeds of Ife Brown with 69 % while 68, 66.8 and 66.5 % were obtained from cultivars IT90K-277-2, IT03K-337-6 and IT96D-610, respectively. The germination capacity of 64 % was recorded from seeds of cultivars IT99K-316-2 while IT07K-299-6 and the remaining cultivars had

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AAG percentages ranging from 53.4 to 62.7 % (Table 1).

For the mixed infection treatments, germination value of 58.6 % was obtained from IT90K-277-2, IT06k-124 and IT07K-292-1-10 BICMV + CPMoV infected cowpea cultivars. This value (58.6 %) was significantly (p<0.05) higher than the values obtained from seeds of other cultivars. Seeds from cultivars IT98K-205-M8, IT97K-499-35, IT06K-137-1 and IT07K-211-1-8 gavegermination values of 56.5, 55, 54.5 and 53.4 % respectively. Seeds of cultivars IT96D-610 and IT00K-901-5 exhibited similar germination percentage of 52 while the remaining cowpea cultivars had germination percentages of between 44.0 and 50.6. Also, seed germinability of 57.3 % was highest in IT07K-292-1-10 with CPMoV + BICMV infected cowpea seeds which was statistically (p>0.05) similar to the performance of seeds of IT97K-499-35 with 56 %. Seeds of cultivar IT04K-267-8 and IT07K-222-2 exhibited 54.6 and 53.7 % respectively, while IT96D-610 and IT04K-291-2 had germination values of 52 % which did not differ from one another. The lowest accelerated ageing germination (AAG) percent of 31.6 was recorded in seeds of cowpea cultivar IT99K-377-1 (Table 1).

DISCUSSION

Germination and longevity are two major indices used for determining the performance capability of seed lot. Seed quality is influenced by the environment where it is produced. Pathogens namely virus, nematode, fungi, bacteria among others are integral components of the environment of any seed crop; failure to effectively manage their competition could mean zero harvest (1). However the imperative of understanding the impact of virus management strategies and management for quality seed production arises from the paucity of information on the agronomy of seed production (1), more so that seed production efforts are judged on the basis of quality of the produce rather than quantity. The result of this study has established a clear negative influence of virus infection on cowpea seed quality and that the differential ranking of the virus infection treatments in different seed quality test is an indication of the response of the developing seeds on the mother plant to competing virus infection situations. Differences in time of flower initiation, pod setting, seed formation and maturity to virus infections are critical factor to tropical farming. The results obtained from this study revealed that there was a variation in

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germination percentage before and after four weeks of storage which is a measure of seed viability and longevity. When seed that has this trait is sown on the field for production, it exhibits a wide variation in performance after sowing due to the differences in quality (1).

It is known that cowpea seedlings are susceptible to virus infection at different stages of development (3). This is supported by the differential responses of cowpea seeds harvested from the different virus treatment seed lots in the present study. The initial general high germination percentage recorded in seeds of all treatment combinations in this study is an indication that the seeds did not exhibit dormancy contrary to what is known with most vegetable seeds when freshly harvested. This rapid germination also showed that the activities of the pathogens (viruses) on the seeds were not severe enough to impaired germination (5). Mandhare and Gawade (17) reported that though seeds obtained from mosaic infected bean at harvest exhibited high seed germination, a significant sharp decline in germination percentage of the seeds was recorded following four weeks of storage at 32 ^{0}C and 50 % relative humidity.

Following storage of seeds for four weeks in this study, a sharp decline in the germination capability of seeds of all the treatment combinations was recorded. This sharp decline in the quality of seeds is abnormal according to the normal/natural seed ageing process (13). The reason may be that the pathogen activities must have been activated which resulted in the sudden and heavy decline in the germination percentages (19). Furthermore, the variation in germination percentages amongst the cultivars and treatments as shown in this study suggest genetic superiority (5) and tolerance level of the cultivars over one another.

CONCLUSION

The results of the experiment revealed that all cultivars were susceptible to single and mixed infections of the two viruses but to seemingly different extent. The germination of seeds as seen from this study was generally high before storage; the high initial germination percentage was not sustained (short lived); an indication that conservation of infected seeds of all cultivars was impaired. More so, all the cowpea cultivars did not exhibit dormancy which is a problem with most freshly harvested vegetable seeds. The benefits of increased

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cowpea production include improved nutrition for humans and livestock, improved soil properties and substantial opportunities for greater income. The monitoring and management of these viruses therefore is crucial to sustainable cowpea production most especially in sub-Saharan Africa. There is the need, therefore, for constant monitoring of legume fields through regular field sanitation, disease surveys to identify new and emerging viruses because these facts present a good starting point for legume virus diseases diagnosis in the study area. Finally, there is also need to ensure availability of acceptable horticultural desirable cowpea cultivars with a high level of resistance to cowpea viruses for the nation to sustain its high level of production.

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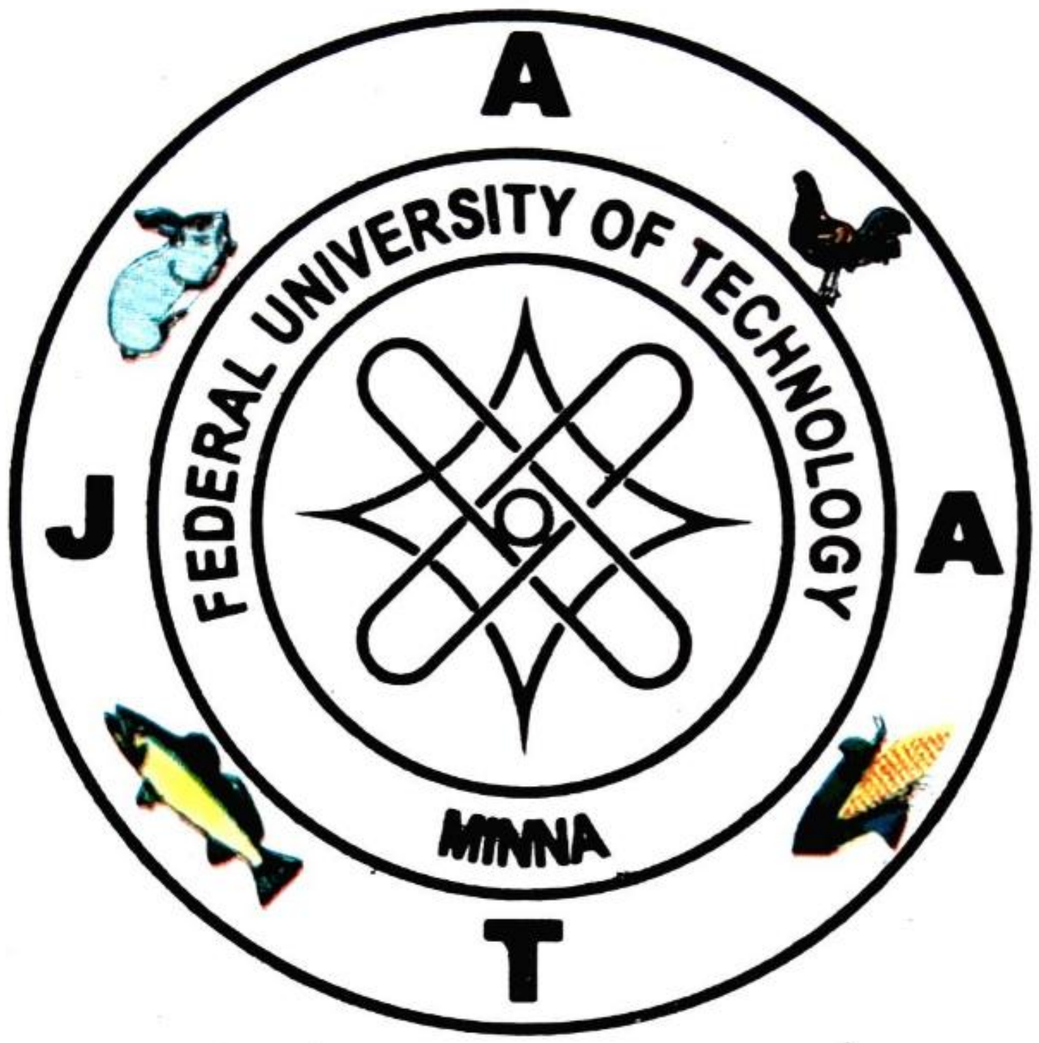

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CURRENT STATUS OF RESEARCH ON *TOMATO SPOTTED WILT* TOSPOVIRUS AND *TOMATO YELLOW LEAF CURL* BEGOMOVIRUS

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ABSTRACT

Tomato spotted wilt virus (TSWV) and Tomato yellow leaf curl virus (TYLCV) are major biotic constraints to several cultivated and wild plants. These viruses belong to the genus tospovirus and begomovirus, respectively. TSWV is about 80-110 nm wide while TYLCV is about 20 x 30 nm in size. Both viruses are difficult to control due to numerous host plants and activities of insect vectors. Incidences and yield losses are usually high. Economic and sustainable management strategies rely on the reduction of inoculum sources, limiting transmission and use of host plant resistance. This paper reviews the current research status on the two viruses. Future research areas are also suggested.

Key words: TSWV, TYLCV, transmission, host range, management

INTRODUCTION

Tomato spotted wilt (Rangaswami and Bagyaraj, 2005) and *Tomato yellow leaf curl* (Fauquet *et al.*, 2008) *viruses* are the most common viral pathogens of tomato and a wide range of cultivated plants world wide. Tomato spotted wilt disease (TSWD) was first reported in Australia in 1919 (Brittlebank, 1919) and by 1925 it had become a serious threat to vegetable crops (Bald and Samuel, 1931) throughout temperate and sub-tropical regions of the world. Incidence of TSWD ranges from 10 to 30 % (Sharman and Persley, 2006) and yield losses may reach 90 % (Cho *et al.*, 1987). *Tomato spotted wilt virus* (TSWV) is transmitted by nine thrips (Thysanoptera:Thripidae) including *Frankliniella fusca* Hinds, *F. intosa* Tryom, *F. occidentalis* Perg., *F. scultzel* Tryh, *F. suchini* Nakahara *et* Monteiro, *Thrips setosus* Moulton, *T. tabaci* Hind, *T. palmi* Karny, and *Scirtothrips dorsalis* Hood (Mound, 2002).

Many wild annual and perennial plants also serve as natural reservoirs and sources of primary inoculants of TSWV (Groves *et al.*, 2003). Symptoms of TSWD on susceptible host plants are concentric ring spots, speckling and chlorotic streaking in oak-leaf patterns on leaves that develop into bud necrosis, stunting, and premature death (Adkins *et al.*, 2006).

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Hitherto, characterization of isolates of TSWV was difficult thus relying solely on symptoms expressed by differential hosts and, when available, by hosts with resistant genes (Best and Gallus, 1953). Verkleij and Peters (1983) used sucrose-gradient centrifugation to separate nucleoprotein and 78 K membrane protein to produce specific antisera to the virus.

At present, immunofluorescence microscopy, enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) are widely used for diagnosis (Assis-Filho *et al*., 2004; Adkins *et al*., 2006), in addition to electron microscopy (Winter *et al*., 2006).

Tomato yellow leaf curl disease (TYLCD) is caused by at least 11 different virus species one of which is *Tomato yellow leaf curl virus* (TYLCV). A member of the family Germiniviridae, TYLCD was first reported on *Lycopersicon esculentum* Mill in Israel (Cohen and Harpaz, 1964). The virus could account for huge quantitative and qualitative losses of 100 % if unchecked.

Thus, incidences and yield losses of 100 % have been reported (Czosnek *et al*., 1990; Pico *et al*., 1996). Typical symptoms induced by TYLCV on susceptible tomato cultivars include reduction in leaf size, yellowing and curling of the leaf, stunting of the plant, and abscission of flowers and fruits (Al- Musa, 1982). TYLCV infects several plants but most pathogenic on tomato crops (Cohen and Antignus, 1964).

The virus also survives in weeds within and outside tomato farms, which are potential sources of virus inoculum for primary and secondary spread of TYLCD (Kashina *et al*., 2002b). The plant species *Achyranthes aspera* L., *Capsicum annum* L., *Datura stramonium* L., and *Nicotiana tabacum* L. are some of the alternative hosts of the virus (Rapisarda, 1990; Kashina *et al*., 2002b).

The virus can be detected in infected plants or vectors through several techniques including Southern blot, squash blot, polymerase chain reaction, and enzyme-linked immunosorbent assay, in addition to the use of electron microscope (Czosnek *et al*., 1988; Kashina *et al*., 2003; Kashina *et al*., 2007b). TYLCV is extensively vectored by aleurodid *Bemisia tabaci* Genn. (Nakhla *et al*., 1978) in a persistent circulative manner (Kashina *et al*., 2007a). A high degree of sequence diversity has been reported among genomes of the virus (Pico *et al*., 1996).

For instance, an Egyptian isolate of the virus was nearly identical to TYLCV isolates from Israel, but more distantly related to those from Thailand and Sardinia (Nakhla *et al*.,

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1993). Abou-Jawadah *et al.* (1999) reported that Lebanese isolate of the virus was closely related to Egyptian, Israeli and Jamaican isolates but not identical to isolates from Sardinia, Spain and Thailand. Additionally, studies have shown that some Tanzania and Uganda isolates of TYLCV were similar to those from Egypt (AVRDC, 1994). Israel and Sardinia (Kashina *et al.*, 2002a).

Management of TSWV and TYLCV in tomato production is very difficult and expensive. Moreover, host resistance to these viruses is not easy to come by (Zhao *et al.*, 1995; Lapidot *et al.*, 1997). Control measures largely rely on reduction of principal sources of inoculum, control of transmission and use of host plant resistance (Ioannou, 1987; Antignus *et al.*, 1995; AVRDC, 1996; Jahn *et al.*, 2000; Gomez *et al.*, 2004). This paper reviews the economic importance, characteristics, transmission, host range and management strategies for these viruses.

CHARACTERISTICS AND SYMPTOMATOLOGY OF TSWV

TSWV is spherical with a diameter of about 80-110 nm and characteristic spikes on its envelope (Francki and Hatta, 1981). Its thermal inactivation point is 45 °C for 10 minutes and dilution - end point varies from 10^3 to 10^4. The pathogen has a longevity *in-vitro*, which ranges between 3 and 6 hours (Rangaswami and Bagyaraj, 2005).

The virus is unique among plant viruses because it is covered by a lipoprotein envelope (Cho *et al.*, 1989). It is a single stranded RNA (ssRNA) member of the family Bunyaviridae (Van Regenmortel *et al.*, 2000; Ullman *et al.*, 2002). This family includes five genera: bunyavirus, phlebovirus, hantavirus, nairovirus, and tospovirus (Elliot *et al.*, 1992). TSWV belongs to the genus tospovirus and is the only genus of the Bunyaviridae that infects plants (Chu *et al.*, 2001).

The tripartite TSWV-RNA genome is made up of small (S) and medium (M) ambisense segments and a large (L) negative strand from five open reading frames (Murphy *et al.*, 1995; Ullman *et al.*, 2002). The small, medium and large segments are approximately 2.9, 4.8, and 8.9 kb in size, respectively. Its S RNA encodes the nucleocapsid (N) and a nonstructural (NS) protein (Kormelink *et al.*, 1991).

Studies have shown that the NS protein is present after translation in both the plant cell and insect vector (Wijkamp *et al.*, 1995). The viral capsid (N) protein plays a significant role in viral replication cycle in a structural and perhaps, regulatory manner through its function in the formation of

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ribonucleoproteins (RNPs) (Adkins, 2000). RNP molecules are encapsidated by multiple copies of the virus encoded N protein to form RNPs (Schmaljohn, 1996). Moreover, a few copies of the viral L protein, which is a putative RNA-directed RNA polymerase are found in connection with RNPs (Adkins, 2000).

Elliot (1996) stressed that RNPs are very important in infection cycle of the TSWV and other bunyaviruses. Since they are not naked viral genomic RNA, they serve as template for both viral gene transcription and genome replication. Earlier, Uhrig *et al.* (1999) studied N protein interactions and postulated that monomers of TSWV interact through hydrophobic forces in a so called head-to tail fashion. Kainz *et al.* (2004) reported a similar result but argued that the head-to tail model was not adequate to account for all aspects of the interaction between N monomers.

Typical symptoms of TSWD include chlorotic or necrotic rings, lines, or spots on leaves, stems, and fruits; necrotic streaks on stems; bronzing, curling, and wilting of leaves, rings, necrotic spots, and malformation on fruits; stunting and necrosis of parts or whole plants, and reduced yield (Agrios, 2004). Rangaswami and Bagyaraj (2005) reported that TSWD symptoms are first seen as slightly bunched appearance at growing points, followed by curling of older leaves. The older leaves turn bronze with brown coloured markings which culminate in irregular patches of dark tissues, particularly on the lower leaves.

The symptoms may spread to cover the whole leaflets and then to leaf stalks. As the disease progresses the infected plants become stunted. Plants can be infected at any growth stage. Attack of young plants may result in death while older plants become stunted with weak shoots.

The infected plants may not produce fruit or they may be of poor quality, with light red, yellow, or white discolourations and characteristic mottling symptoms. Symptoms of TSWD vary greatly with the host affected, plant organ affected, age of plant at infection (Agrios, 2004), and environmental conditions (De La Torre *et al.*, 2002).

TRANSMISSION AND HOST RANGE OFTSWV

The virus is transmitted exclusively by thrips in a propagative manner (Persley *et al.*, 2006). TSWV is not ovarially transmitted (Assis-Filho *et al.*, 2003) and seed transmission has never been reported (Reddy and Wightman, 1988). Although mechanical transmission of the virus is

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feasible under controlled conditions, such transfers are uncertain in the field (Bald and Samuel, 1931).

Tospoviruses are currently vectored by nine species of thrips but *F. occidentalis* and *F. fusca* are the most important (Riley and Pappu, 2000). However, while *F. occidentalis* is an efficient vector in tomato plants, TSWV incidence in tobacco has been associated with *F. fusca* population (Riley and Pappu, 2004). In another investigation, Joost and Riley (2004) observed a relatively high density of *F. fusca* in pre-blossomed tomato plants. Recently, Assis-Filho *et al.* (2005) detected the virus in *F. tritici*. TSWV is acquired by first instar thrips feeding on infected host (Assis-Filho *et al.*, 2005).

The minimum period for virus acquisition is 15 minutes but efficiency of transmission increases with feeding period.

The virus enters the midgut epithelial cells, replicates, moves to salivary glands during pupation, and is transmitted over the entire life of an infected adult (Assis-Filho *et al.*, 2002; Nagata *et al.*, 2002).

Additionally, studies have shown that TSWV acquired by *F. tritici* replicated and moved within the alimentary canal of the insect *F. occidentalis* but the virus was not found in the salivary glands, a condition for virus transmission.

Therefore, *F. tritici* is not yet an established vector of TSWV (Assis-Filho *et al.*, 2005). Moritz *et al.* (2004) reported that TSWV can invade the salivary glands of *F. occidentalis* when the brain of the first instar larvae is displaced out of the head and the cells of the foregut and salivary glands are in close contact. Thrips can only acquire the virus in the relatively immobile larval stage (Bald and Samuel, 1931), successful vectors must be able to develop to the adult stage on the attacked plant or another near-by plant after acquiring the pathogen.

Moreover, successful vectors must feed on susceptible healthy plant before the virus can be spread. After transmission, the ability of the virus to multiply in its host and produce visible symptoms is influenced by the plant genotype, plant age, and climatic conditions (Best, 1968).

Therefore, a sound knowledge of thrips dynamics is essential for better understanding TSWV epidemiology. For example, Bailey (1938) observed an increase in thrips population in infested fruit orchard when reduced rainfall and high temperatures accelerated drying of a non-irrigated wild host. Earlier, Bald (1937) documented that temperatures above 23.8°C were most favourable for disseminating *F. schultzei* while low temperatures suppressed adult activity and prolonged development time. Also, Harding (1961) noted that thrips

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migration declined with heavy rainfall and low temperatures.

TSWV has an extensive host range (Peters, 1998). The virus is hosted by over 650 plant species including important crops such as *Arachis hypogaea* L., *Capsicum annum* L., *Solanum tuberosum* L., *Nicotiana tabacum* L., *L. esculentum*, and *Apium graveloens* L. (Best, 1968).

Several workers have reported that these wild and cultivated plants serve as natural reservoir and sources of primary inoculants (Yudin *et al.*, 1988; Toapanta *et al.*, 1996; Agrios, 2004). Trichilo and Leigh (1988) stated that the thrips vectors of the pathogen are polyphagus and so frequently come in contact with several host plants of differing suitability for reproduction. Thus, the suitability of a particular female vector influences survivorship and transmission fitness of her offspring (Ullman *et al.*, 2002).

Groves *et al.* (2001) observed that *Stellaria media*, *Scleranthus annuus*, and *Sonchus asper* weeds differed greatly in their ability to habour *F. fusca* and sources for subsequent spread of TSWV in spring. This was attributed to differential susceptibility among vegetative and flowering stages of the weeds. Therefore, incidence of the virus would be greatest in those plants that are in the most susceptible stage during flight of viruliferous thrips (Burdon *et al.*, 1989).

MANAGEMENT OF TSWD

Control of tospovirus is difficult because of its wide host range and thrips vectors (Agrios, 2004). Sustainable management strategies include rouging of infected plants, use of TSWV-free planting materials, elimination of weed hosts and biological control (Robb, 1989; Ochoa *et al.*, 1996; Loomans *et al.*, 1997; Funderbunk *et a.*, 2000; Maris *et al.*, 2003).

However, integrated control measure involving cultural practices and host plant resistance are currently the most effective option (Johnson *et al.*, 1996). Insecticide control of its vectors has not been successful due to development of resistance to the same (Zhao *et al.*, 1995). Additionally, the high cost and various health hazards associated with its use limit its acceptability (Maris *et al.*, 2003).

Consequently, search for alternative control measures are being investigated. For example, Devi *et al.* (2004) investigated and successfully used the extracts of *Mirabilis jalapa* and *Harpulia cupanioides plants* to contain the virus. Resistance gene has been found in chrysanthemum, lettuce, pepper and tomato (Steven *et al.*, 1992; Boiteux and De-Avila, 1994; Cho-Custer *et al.*, 1996; Daughtrey *et al.*, 1997; Ceholla-Cornejo *et al.*, 2003). Moreover, appreciable level of

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resistance has been reported to its insect vectors in cabbage and groundnut (Kinzer *et al*., 1973; Broadbent *et al*., 1990; Fery and Schalk, 1991; Rhoda *et al*., 1991; Kumar *et al*., 1995; Kogel *et al*., 1998; Maris *et al*., 2002).

Some typical examples are the *Capsicum chinense* Jacquin accession PI 152225, 159236 and Panca (Syn. CNPH 275) (Moury *et al*., 1997). The resistance is expressed as a hypersensitive response and is being controlled by a dominant gene *Tsw*.

In groundnut, a significant level of resistance has been reported in GA T-2846 and Robut 33-1 to the pathogen (Amin, 1985; Culbreath *et al*., 1996).

However, some resistance-breaking isolates of the virus have emerged in Brazil, Italy, Spain and Louisiana (Hobbs *et al*., 1994; Boiteux and Nagata, 1992; Roggero *et al*., 2002; Margaria *et al*., 2004).

Furthermore, it has been confirmed that the use of thrips-resistant genotypes might affect TSWV transmission negatively or positively. For example, Van de Wetering (1999) reported an increased virus spread on a thrips-resistant chrysanthemum.

This was attributed to the altered feeding behaviour of *F. Occidentalis*.

CHARACTERISTICS AND SPMPTOMATOLOGY OF TYLCV

TYLCV is a member of the geminiviruses with characteristics circular single stranded DNA (ssDNA) genome, of 2.8 kb encapsidated in geminate particles and is about 20 x 30 nm in size (CABI, 1990). There are three genera (begomovirus, curtovirus, and mastrevirus) in the Geminiviridae family with similar genomic structure (Mayo and Pringle, 1998).

The virus' genome encodes six open reading frames (OFRs), two on the virion (+) strand including the capsid protein, and four on the complementary (-) strand consisting of the *Rep gene* necessary for TYLCV replication (Czosnek, *et al*., 1994).

However, genomic differences are possible among isolates from different regions of the world. Antignus and Cohen (1994) carried out a complete nucleotide sequence of a mild Israeli isolate of TYLCV and found a sequence of p TY 2.8 which was almost identical in OFRs, the putative coat protein gene (VI), V2 and *Rep genes* to the previously described severe Israeli TYLCV isolate. Conversely, nucleotide sequence intergenic region, the putative replicase, ORF and *Rep gene* of the mild isolate have 78, 87, and 76 % homology, respectively, compared with the previously described

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severe Israeli isolate. Sequence analysis of TYLCV isolates from Israel, Italy and Thailand revealed that the virus is unusually heterogeneous (Keyr-Pour *et al.*, 1991). Intergenic region is a reliable indicator of the relationship among Geminiviruses and isolates of the same strain usually have intergenic region nucleotide sequence identity greater than 90 %. Moreover, Fauquet *et al.* (2008) reported that 11 different virus species associated with TYLCD could be distinguished based on nucleotide identity differences.

TYLCV-infected plants exhibit marked stunting, branches and petioles tend to assume an erect position, leaflets are upward and inward, revealing severe interveinal chlorosis, small leaf size, flower abortion, reduced fruit set and infected young plants produce almost no marketable yield (Pilowsky and Cohen, 1990).

TRANSMISSION AND HOST RANGE OF TYLCV

TYLCV is vectored mainly by the aleurodid whitefly (*B. tabaci*) in a persistent manner. TYLCD incidence and whitefly population are positively correlated (Cohen and Nitzany, 1966; Cohen and Antignus, 1994; Sanchez-Campos *et al.*, 2000). Studies have shown that the virus can be passed transovarially for at least two generations and through moulting (Cohen and Nitzany, 1966; Ghanim *et al.*, 1998; Bosco *et al.*, 2004). Following acquisition by the insect vector, the virus (DNA and infectivity) remains associated with the vector throughout its lifetime (Rubinstein and Czosnek, 1997).

Female *B. tabaci* are more efficient vectors than males (Cohen and Nitzany, 1966). The minimum acquisition access period (AAP) and inoculation access periods (IAP) are approximately 10 to 20 min. However, the rate of transmission increases with long AAPs and IAPs. The minimum latent period varies from 28 48 h and the maximum latent period is 48 h (Ioannou, 1985; Brown and Nelson, 1988; Mansour and Al-Musa, 1992).

The virus persists in the vector for 11 -12 days (Kashina *et al.*, 2007a) but it does not replicate in it (Cohen and Nitzany, 1966; Ioannou, 1985). The wide range of value is an indication of the efficiency with which a given virus establishes a systemic infection in a plant rather than differences in the velocity of translocation in the vector. Ghanim *et al.* (2001) observed that in spite of the female *B. tabaci* higher efficiency of transmission of TYLCV than males; the virus was detected in the salivary glands of both after approximately the same AAP. Mehta *et al.* (1994) observed that the minimum latent period for the closely

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related strains from Egypt was 24 h while Caciagali *et al*. (1995) reported 17 h for the distant TYLCV from Sardinia.

The efficiency of TYLCV transmission by *B. tabaci* is influenced by the vector's fitness, and that is a function of the physiological condition of the source plants. A TYLCV susceptible tomato cultivar could be prone to high risk of virus after infection. However, as the infected plants deteriorate due to expression of disease symptoms, their ability to act as virus source declines. Conversely, a field of moderately resistant tomato cultivars such as 84874 will serve as an effective reservoir of the virus throughout the season, because they do not deteriorate as much as the former (Lapidot *et al*., 2001).

These researchers further elucidated that plants exhibiting a high level of resistance to the virus pose the lowest risk of TYLCD epidemics. TYLCV can be transmitted by grafting (Ioannou, 1985) but seed and mechanical transmission have not been successful (Brown and Nelson, 1988; Kashina *et al*., 2007a).

Various anatomical and immunolocalization studies have indicated that geminivirus particles are probably ingested along the phloem sap of infected tissues through the stylets, the esophagus and finally into the filter chamber. Virions are then conveyed through the gut wall into the haemocoel and finally to the salivary glands. The virus is translocated into salivary duct and then excreted during feeding (Harris *et al*., 1995; Hunter, 1998; Ghanim *et al*., 2001).

Investigating the route of the virus, Ghanim *et al*. (2001) first detected it in the head of *B. tabaci* after a 10 min. AAP, in the midgut after 40 min., and was first observed in the haemolymph after 90 min. Furthermore, the virus was detected in the salivary gland 5.5 h after it was first noticed in the haemolymph.

Several wild and cultivated plants have been reported as alternative hosts of TYLCV. In Cyprus, the plants that serve as natural hosts of the virus include *D. stramonium*, *L. esculentum*, *N. tabacum*, and wild *Lycopersicon* spp. (Ioannou, 1987; Rapisarda, 1990).

In Israel and Jordan *D. stramonium*, *Lens esculenta* Moench, *Malva nicaensis* A.II., *M. parviflora* L., *N. tabacum*, and *Phaseolus vulgaris* L. have been reported as its natural hosts (Cohen and Nitzany, 1966; Nitzany, 1975; Al-Musa, 1986; Cohen *et al*., 1988; Mansour and Al-Musa 1992). Nono-Womdim *et al*. (1996) reported *Achyranthes aspera*, *Euphorbia heterophylla* and *Nicandra physaloides* as natural hosts of TYLCV in Tanzania. Additionally, Kashina *et al*. (2002b) found the weed species

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Achanthospermum hispidum DC, *Amaranthus spinosus* L., *A. viridis* L., *Bidens pilosa* L., *Boerhavia diffusa* L., *Cassia occidentalis* L., *Chromolaena odorata* (L.) R. M. King & H. Rob., *Commelina erecta* L., *Eclipta prostrata* (L.) L., *Erigeron floribundus* (Kunth) Sch. Bip., *Ipomoea batatas* L., *Physalis angulata* L., *Portulata retusa* Engelm., *and Sida acuta* Burm. f. as experimental hosts of the virus in Tanzania.

MANAGEMENT OF TYLCV

Management of TYLCV is very difficult, expensive, and has limited options (Lapidot and Friedmann, 2002). Farm sanitation, which involves the clearing of weeds, debris of tomato plants and other solanaceous crops in which the virus has overwintered is adopted by tomato farmers. This measure is also effective in restricting the migration of viruliferous whiteflies (Ioannou, 1987; Cohen *et al.*, 1988). Also, the use of virus free planting materials has been recommended. This is vital because early appearance of TYLCD with its attendant high yield loss is aggravated if infected seedlings are transplanted (Kashina *et al.*, 2002b). The young infected plants serve as primary sources for secondary spread of the virus to healthy plants.

Manipulation of sowing date is another strategy being used to eradicate the threat posed by the disease. For instance, planting may be delayed in order to avoid periods of peak vector populations, which often occurs after periods of high temperature and low relative humidity (Ioannou and Iordanou, 1985).

Interplanting tomato plants with other crops such as cucumber, eggplants and peppers is another strategy that has recorded some level of success. The practice is effective in diverting the whiteflies from tomato to other preferred hosts, especially if the latter is planted earlier than tomatoes (Al-Musa, 1982). Mulching of the seed beds prior to transplanting of tomato seedling has been employed to delay TYLCV infection (Cohen *et al.*, 1974) for at least two weeks by discouraging vector landing on the crops. Antignus *et al.* (1996) reported that ultraviolet absorbing plastic sheets and screens can be used to reduce penetration of whiteflies into covered greenhouses.

Biological control using predators or parasites *Encarsa formosa*, *E. lutea* and *Eremocerus mundus* has been successfully used to control the insect vector and virus spread in the Mediterranean regions. However, a sound knowledge of the delicate interaction between whitefly and its natural enemies is a prerequisite; otherwise, indiscriminate use of the agents of control can disrupt this balance (Natarajan, 1990; Henneberry and Bellows, 1995).

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The spread of TYLCD can be partially curtailed by spraying insecticides against its vector (Cohen *et al*., 1974; Berlinger and Dahan, 1989). The partial effect of insecticides is due to the low sensitivity of whitefly, its ability to develop resistance against them, and possible deleterious effect on the environment (Dittrich *et al*., 1990; Pico *et al*., 1996). Other difficulties associated with the use of insecticides include simultaneous presence of different developmental stages of vector population among neighboring fields (Al-Musa, 1986). Lacasa and Contreras (1995) reported that some level of success was achieved in reducing the vector population when insecticide spray coincided with the early stages of insect development, but the strategy failed to reduce incidence of the disease as the number of insects required for field epidemics is often very low, and the transmission efficiency is very high.

The use of resistant genotypes seems to be a promising approach for TYLCV control. Resistance to the virus has been found in wild relatives of the cultivated tomato (Geneif, 1984; Kasrawi *et al*., 1988). Earlier studies on breeding for resistance to the pathogen began at the Volcani Center in Israel in 1974, using accession LA 121 of *Lycopersicon pimpinellifolium* (Jusl.) Mill as a donor of resistance gene (Pilowsky and Cohen, 1974). However, TYLCV control was accompanied with marked reduced growth and yield. Therefore, a new breeding programme was put in place in 1977 to incorporate tolerance from accession PI 126935 of *L. peruvianum* (L.) Mill., resulting in the development of F_1 hybrid TY 20 (Pilowsky *et al*., 1989).

This cultivar exhibited delayed symptoms and accumulation of viral DNA (Rom *et al*., 1993). Advanced breeding lines with high levels of resistance derived from several wild *Lycopersicon* spp. have been developed and are now widely utilized in the breeding of desirable F_1 hybrids (Lapidot *et al*., 1997; Friedmann *et al*., 1998). Pilowsky and Cohen (1990) reported that tolerance to TYLCV was inherited as recessive trait. Resistance of plants to the virus is controlled by five recessive genes (Pilowsky and Cohen, 1990). However, Kasrawi (1989) indicated that resistance to TYLCV is conditioned by a single dominant gene.

Abou Jawdah *et al*. (1999) found in the field and screenhouse experiments that the tomato cultivars TY Carla, PSR and RS lines were resistant and also exhibited determinate growth while S & G 143 and the DR lines were resistant with semi determinate growth, respectively. Earlier, Abou Jawdah *et al*. (1996) found the wild accession *Lycopersicon chilense* LA 1969 to be resistant to the virus.

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The tomato lines LD 3, LD 4, LD 5, and LD 6 were resistant to TYLCV while 'ARO 8479' and 'HA 3108' were tolerant to it (Gomez *et al.*, 2004).

Furthermore, Kashina *et al.* (2004) reported that the tomato cultivar TY 172 was resistant to the virus in Tanzania.

Atabekov and Dorokhov (1984) reported that inhibition of virus accumulation and/or virus short and long distance movement are among the most conspicuous mechanisms of plant virus resistance. Ber *et al.* (1990) observed that TYLCV DNA in susceptible plants translocated from the inoculated youngest leaf to the four and five upper leaves and finally to the roots, the same route followed by assimilates.

Conversely, movement in tolerant plants was limited to the second leaf and to the shoot apex which was probably due to restricted rate of cell to cell movement in the tolerant lines. Michelson *et al.* (1994) compared two nearly isogenic lines (susceptible and tolerant), which differed only in a single mapped chromosomal segment and found that TYLCV DNA rarely accumulated in leaves of the tolerant line 52; when the level of inoculum was high, significant amounts of viral DNA were observed, but it accumulated at a rate slower than that in susceptible line 50.

Most of the techniques used for genetically engineered resistance to begomoviruses are based on the replication associated protein (*Rep*) sequence. Yang *et al.* (2004) evaluated TYLCV resistance under field conditions using different constructs of the TYLCV replication associated protein (*Rep*) and C4 gene sequences and recorded the best resistance in the constructs containing intergenic region (IR) and 2/5 *Rep gene* sequences of the virus in either the sense or ambisense orientation. Also, resistance was observed at high frequency in both the R_1 and R_2 generations.

CONCLUSION AND FUTURE RESEARCHAREAS

TSWV and TYLCV are prevalent the world over. Although market demand for tomato continues to increase productivity is not justified by the ever increasing cultivated land area. Intensive studies have been conducted to investigate the epidemiology and survival of these viruses and different strategies have been used to manage them with varying levels of success. Since it is not feasible to use a single strategy to achieve absolute control of the viruses, integration of the strategies highlighted in this review can be exploited as a veritable tool for sustainable management of the same. However, future research should focus on identification of the various strains in each

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agro-ecological zone with a view to breeding cultivars with multiple resistance to them. Additionally, more information on the alternative hosts of these viruses is essential in each country where the diseases occur.

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