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1 **Storage root yield of sweetpotato as influenced by sweetpotato leaf curl virus and its**
2 **interaction with sweetpotato feathery mottle virus, and sweetpotato chlorotic stunt virus in**
3 **Kenya**

4

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27

28 **Abstract**

29 The effect of a Kenyan strain of sweetpotato leaf curl virus (SPLCV) and its interactions with
30 sweetpotato feathery mottle virus (SPFMV), and sweetpotato chlorotic stunt virus (SPCSV) on
31 root yield was determined. Trials were performed during two seasons using varieties contrasting
32 in their resistance to sweetpotato virus disease, 'Kakamega' and 'Ejumula', in a randomized
33 complete block design with sixteen treatments replicated three times. The treatments included
34 plants graft inoculated with SPLCV, SPFMV and SPCSV alone and in possible dual or triple
35 combinations. Yield and yield related parameters were evaluated at harvest. Results showed
36 marked differences in the effect of SPLCV infection on the two varieties: 'Ejumula', which is
37 susceptible to SPFMV and SPCSV, suffered no significant yield loss from SPLCV infection,
38 whereas 'Kakamega', which is more resistant to SPFMV and SPCSV, suffered an average of
39 47% yield loss, despite only mild symptoms occurring in both varieties. These results highlight
40 the variability in sensitivity to SPLCV between sweetpotato cultivars as well as a lack of
41 correlation of SPLCV related symptoms with susceptibility to the virus. In addition, they
42 underline the lack of correlation between resistance to the RNA viruses SPCSV and SPFMV and
43 DNA virus SPLCV.

44 **Key words**: SPLCV, sweepovirus, SPFMV, SPCSV, treatment, yield

45 **Introduction**

46 Ranked seventh in global food crop production, Sweetpotato (*Ipomoea batatas*) is the third most
47 important root and tuber crop after potato and cassava. In the developing world, it ranks fourth in
48 importance after rice, wheat, and corn (Kays, 2005). It is one of the traditional crops that play an
49 important role in addressing food insecurity in most rural households in Africa (Gruneberg et al.
50 2015). Orange-fleshed sweetpotato varieties' high β-carotene (source of pro-vitamin A) has seen
51 an increased utilization in food and dietary programs aimed at addressing vitamin A deficiency;
52 a global challenge in Sub-Saharan Africa (SSA) (Kurabachew, 2015). The crop is cultivated all-
53 round the year, producing high yields under marginal conditions. Sweetpotato yields differ, from
54 over 25 metric tons per hectare with high-input to below 3 metric tons per hectare when grown
55 as a subsistence crop with minimal input (Ling et al. 2010). In Kenya, sweetpotato production is
56 hindered by numerous biotic, abiotic and social factors (Kivuva et al. 2015). Pests and diseases
57 are the greatest limitation that affect production and reduce yields (Motsa et al. 2015). Viral
58 diseases are the greatest threat to sweetpotato production, causing yield losses of up to 80%
59 (Gibson and Kreuze, 2015).

60

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61 Recycling of vine cuttings leads to a significant decline in root yield and quality due to virus
62 accumulation. Sweetpotato virus disease (SPVD), caused by dual infection of a Potyvirus, sweet
63 potato feathery mottle virus (SPFMV), and a Crinivirus, sweetpotato chlorotic stunt virus
64 (SPCSV), is most devastating in East Africa (Karyeija et al. 1998; Gibson et al. 1997). SPFMV
65 is common in sweetpotato producing regions around the world (Ateka et al. 2004). SPCSV
66 induces synergistic interactions with other sweetpotato viruses blonging to the species
67 sweetpotato mild mottle virus (SPMMV) (Tairo et al. 2005), sweetpotato virus G (SPVG) (IsHak
68 et al. 2003), and cucumber mosaic virus (CMV) (Cohen and Loebenstein, 1991). However, yield
69 decline attributable to these viruses is cultivar dependent and previous studies have given
70 contradictory findings. Milgram et al. (1996) and Clark and Hoy (2006), reported that single
71 infection with SPFMV, SPVG, or isolates of the species sweetpotato virus 2 (syn. Ipomoea vein
72 mosaic virus) did not considerably affect yield. In the contrary, Gutierrez et al. (2003) found that
73 SPFMV-infected plants produced better yield than the healthy control. On the other hand,
74 Gibson et al. (1997), Mukasa (2004), Njeru et al. (2004), Domola et al. (2008), reported yield
75 reduction of up to 46%.

76 To date, over 30 viruses have been characterized as pathogens of sweetpotato, half of them
77 belonging to the families *Geminiviridae* and *Caulimoviridae* (Clark et al. 2012). Members of the
78 species sweet potato leaf curl virus (SPLCV) and related viruses infecting sweetpotato, belong to
79 the genus *begomovirus* in the family *Geminiviridae*. They are highly variable making their
80 taxonomy, which has been revised over recent years, problematic, but they can be distinguished
81 from begomoviruses infecting other crops by their phylogenetically unique lineage, referred to as
82 sweepoviruses (Albuquerque et al. 2012; Esterhuizen et al. 2012; Fauquet and Stanley, 2003;
83 Wasswa et al. 2011, Cuellar et al. 2015). We will refer to them as such in this manuscript when
84 discussing them in general, rather than individual isolates. Sweepoviruses are transmitted
85 through vegetative propagation and semi-persistently by whiteflies (*Bemisia tabaci*). They have
86 been isolated from sweetpotato fields in different parts of the world including the United States,
87 South America, the Middle East, Southeast Asia, and East Africa (Briddon et al. 2006; Luan et
88 al. 2006; Miano et al. 2006; Prasanth and Hegde 2008; Lozano et al. 2009; Paprotka et al. 2010;
89 Albuquerque et al. 2012; Wasswa et al. 2011). Sweepovirus infected plants may exhibit upward
90 curling and/or rolling of leaves, vein swelling, and vein mottle in young sweetpotato plants.

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91 However, symptom remission is observed in mature plants, and most plants become
92 symptomless (Miano et al. 2006). Sweepovirus single viral infections often lack obvious
93 symptoms making it difficult to be recognized by growers. Miano et al. (2006) reported the
94 occurrence of sweepoviruses in an agricultural field station in Kenya. Countrywide surveys
95 conducted in 2011 (Maina, 2014; Maina et al. 2017) and (Wanjala, 2016/2017 - *unpublished*
96 *data*) confirmed sweepoviruses to be present in the major sweetpotato growing regions of the
97 country. The presence of sweepovirus inoculum in major sweetpotato producing areas in Kenya
98 and the continuing expansion of the vector - *Bemisia tabaci* (Simmons et al. 2008) might have
99 contributed to its broad geographic distribution in Kenya.

100 Despite the lack of characteristic foliar symptoms, sweepoviruses have been reported to cause
101 between 10 and 80% yield loss for different sweet potato cultivars (Clark and Hoy 2006; Ling et
102 al. 2010; Gibson and Kreuze, 2015). Studies have demonstrated that sweepoviruses when co-
103 infected with SPCSV can lead to increased viral titres and symptoms in sweetpotatoes under
104 controlled conditions (Cuellar et al. 2015). However, limited knowledge exists on the interaction
105 of sweepoviruses, SPFMV and SPCSV in sweetpotato under field conditions or their effect on
106 yield and quality of sweetpotato roots. Therefore, the study aimed at evaluating the effect of
107 Kenyan isolates of a sweepovirus (SPLCV), SPFMV, and SPCSV alone, and co-infections on
108 sweetpotato root yield of two cultivars contrasting in their resistance to SPVD.

109

110 **Methods and Materials**

111 **Sources of healthy planting and detection of sweetpotato viruses**

112

113 Clean virus tested (VT) *in vitro* planting materials were obtained from the International Potato
114 Centre (CIP) germplasm collection at Kenya Plant Health Inspectorate Services - Plant
115 Quarantine and Biosecurity Station (KEPHIS-PQBS) Muguga, Kenya. Plantlets of varieties
116 'Kakamega' and 'Ejumula' were hardened in insect proof greenhouses and away from plants that
117 might be infected with viruses. Both cultivars are landraces, widely adaptable, have good storage
118 root shapes if grown in light soils, high dry matter content, and excellent consumer acceptance,

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119 especially among children and women (Mwanga et al. 2007). Ejumula is susceptible to SPVD
120 while Kakamega shows levels of field resistance to sweetpotato virus disease.

121 Biological indexing was carried out as described by Dennien et al. (2013) on *Ipomoea setosa*
122 (indicator plant) that is highly sensitive to most sweetpotato infecting viruses. Vines singly
123 infected with SPFMV, SPFMV and SPLCV were used as scions to an *Ipomoea setosa* stock
124 seedling following the procedures in (Beetham and Mason 1992 and Dennien et al. 2013). Virus
125 infection treatments (T1–T16) are described in Table 1. *I. setosa* seedling was grown out to 10
126 nodes (4-6 weeks after planting) and grafted with 2 two-node scions from the test plant, one from
127 the basal portion of the vine and one from near the apex of the vine. A wedge graft was made at
128 about 3 nodes above the cotyledonary node and a side veneer graft just below the cotyledonary
129 node. Grafted plants in the pots were covered with plastic bags and placed into large, shallow
130 trays lined with plastic sheeting. The *I. setosa* indicator plant was allowed to grow. To capture
131 transient symptoms, indicator plants are observed twice weekly until 21 days post grafting (PG),
132 then weekly until 42 days PG. The I. setosa was cut back above the graft site and allowed to
133 regrow for an additional 3-4 weeks, continually observing for symptom development. Symptoms
134 typical of different viruses as illustrated in Clark et al., 2012 and Dennien et al., 2013 were
135 recorded.

136 A standard Nitrocellulose membrane enzyme-linked immunosorbent assay (NCM- ELISA) was
137 done using a test kit manufactured by the International Potato Center and as and described by
138 Dennien et al (2013). It tests for 10 known sweetpotato infecting viruses: (C-6, CMV, SPCaLV,
139 SPCV, SPCFV, SPCSV, SPFMV, SPLV, SPMMV, SPMSV and SPVG). It is a prerequisite for
140 the test to use material that is first grafted onto I. setosa. This increases the virus concentration in
141 the indicator and prevents inhibitors present in sweetpotato sap. There are no antisera available
142 for SPLCV and sweepoviruses were tested by PCR as described by Li et al. (2004); using
143 Sweepovirus-specific primers SPG1 (5'-CCC CKG TGC GWR AAT CCA T-3') and SPG2 (5'-
144 ATC CVA AYW TYC AGG GAG CTA A-3'), designed to amplify a 901-bp region
145 encompassing partial AC1 and AC2 open reading frames (ORFs).

146 **Source of virus inoculum and virus inoculation**

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147 Plants singly infected with SPCSV (isolate KE_4) and SPFMV (isolate KE_42). used for graft
148 infection were obtained from KEPHIS-PQBS. Sweepovirus (SPLCV) isolate KE_97 positive
149 plants were collected in different parts of Kenya during surveillance surveys. Viruses were
150 confirmed by grafting to *I. setosa* and use of NCM ELISA. In addition, the plants were subjected
151 to screening by PCR for begomovirus as described above by Li et al. (2004). SPCSV and
152 SPFMV were tested with Reverse Transcription PCR (RT-PCR) as described by Kwak et al.
153 (2014). Furthermore, local strains of sweepovirus positive samples were confirmed by Sanger
154 sequencing of the PCR product (GenBank id MN122257) and confirmed isolate KE_97 was a
155 sweepovirus most closely related to SPLCV and we will refer to it as SPLCV from here
156 onwards. Two-node cuttings were obtained from the VT hardened mother plants of 'Kakamega'
157 and 'Ejumula' and established in a three-liter pot: 17 cm diameter and 20 cm height. Media
158 consisted of sterile top forest soil: cow manure: gravel at a ratio of 5:2:1. Plants were grown in
159 the greenhouse at an average temperature of 28° C and watered as needed. After one month when
160 the plants were ~30 cm tall, 20 plants were graft-infected with 5 cm stem scion using side-veneer
161 procedure (Hartmann et al. 1997) on both 'Ejumula' and 'Kakamega'. **Table 1 shows** the
162 different combinations of virus infections with SPLCV, SPFMV, and SPCSV, alone and in
163 possible dual combinations used as treatments in this study. Different treatments were kept in
164 separate insect-proof chambers in the greenhouse to avoid cross-infection.

165

166 **Greenhouse multiplication of planting material inoculated with viruses**

167 The different treatments (T1–T16 described in **Table 1**) were tested at three months after
168 inoculation, by Quantitative Reverse Transcription PCR (qRT-PCR) to confirm the
169 presence/absence of SPFMV, SPCSV and SPLCV. qRT-PCR reactions were carried out as
170 described by Cuellar et al. (2015), for SPFMV the primers 5'-CGC ATA ATC GGT TGT TTG
171 GTT T-3' and 5'-TTC CTA AGA GGT TAT GTA TAT TTC TAG TAA CAT CAG-3', and the
172 probe 5'-[6-FAM]-AAC GTC TCC ACG CAA GAA GAG GAT GC-[TAMRA]-3' were used
173 corresponding to the coat protein region of the genome. For SPLCV the primers 5'-GAG ACA
174 GCT ATC GTG CC-3' and 5'-GAA ACC GGG ACA TAG CTT CG-3', and the probe 5'-6FAM-
175 TAC ACT GGG AAT GCT GTC CCA ATT GCT-TAMRA-3' were used corresponding to ACI

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176 fragment of coat protein as described by Ling et al. (2010). Plants that tested positive as expected
177 were rapidly multiplied in seedling trays to generate enough material for field trials. During
178 multiplication, a new sterile scalpel blade was used to cut scions to avoid cross contamination
179 between treatments. To ensure that adequate planting material was available for field
180 experiments, plants with double/multiple viruses were multiplied in extra trays due to
181 slow/stunted growth. The multiplied planting material was further randomly tested by qRT-PCR
182 to confirm their infection status before planting in the field.

183

184 **Field experimental design**

185 Field trials were conducted for two seasons at the Kenya Agricultural and Livestock Research
186 Organization, Kiboko Centre, Makueni County in Kenya. The Centre is situated at Latitude S 02
187 ° 12.781', longitude E 037 ° 43.078' and 931 meters above sea level. The soils were sandy loams
188 for each trial in both seasons. Mean annual rainfall in the region is 50 mm with mean monthly
189 maximum temperature of 33 ° C. The two seasons of planting were three months apart. The first
190 field trial was established in September 2017 to February 2018 while season II was set up in
191 December 2017 to May 2018. Both trials were laid using a randomized complete block design
192 (RCBD) with three replicates for the sixteen treatments. The land was ploughed, harrowed and
193 ridges prepared by hand at the two sites before planting. Each replicate (plot) comprised 40
194 plants at inter and intra-row spacing of 1 m and 0.3 m, respectively. Vine cuttings were four
195 weeks old (~ 30 cm long) at the time of planting. Plants were watered immediately after planting
196 and watered by overhead irrigation for 3 hours at night every four days. Weeding was done
197 manually using hand hoes twice a month in the first two months and once thereafter until the
198 crop was harvested. Two rows of finger millet were planted between each plot to reduce spread
199 of viruses between plots by insect vectors. To monitor whitefly abundance, a yellow sticky card
200 trap (26 cm^2) was placed horizontally at canopy height at the center of each plot. These were
201 replaced after every two month. To minimize further spread of viruses between plots; plants
202 were sprayed fortnightly by alternating systemic and contact insecticide as described by
203 manufacturer on the container product label.

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204 **Evaluation of SPLCV, SPFMV and SPCSV under field conditions.**

205 Disease symptom evaluation was done at 30, 90 and 120 days after planting as described by
206 Hahn et al. (1981). A severity score of 1–5 was used, where 1 = plants showing no symptoms; 2
207 = virus symptoms just starting to appear and this can be as mild chlorotic spots on the older
208 leaves or mild vein clearing or mild purpling at the leaf margin of mature leaf; 3 = the symptoms
209 in 2 enlarge and become more visible; 4 = infected plants showing severe disease symptoms
210 including leaf purpling, leaf chlorosis and leaf shape starts to get distorted; and 5 = infected
211 plants showing very severe virus disease symptoms including total distortion in leaf shape,
212 stunted growth, mosaic, leaf chlorosis and sometimes complete death of an infected plant. At the
213 end of both growing seasons, cross contamination between plots was evaluated by testing with
214 RT-PCR. Three leaves (third/fifth/seventh) were collected from ten plants in the inner middle
215 rows, placed between filter papers and put in a ziplock bag with silica gel. Silica gel was
216 changed several times when the color changed from blue to pink to ensure that the leaves were
217 well desiccated. Samples were pooled into one per plot and tested for SPCSV, SPFMV (RT-
218 PCR) and SPLCV (PCR) as described above to check if any cross contamination of viruses had
219 occurred between plots.

220

221 **Root yield assessment**

222 Plants were harvested at 150 days after planting (DAP); 15 February 2018 and 15 May 2018,
223 respectively. Storage roots were graded as marketable (good-quality roots of 100–1200 g) or
224 unmarketable (<100 g). Sixteen parameters were collected during the experiment. These
225 included: disease severity, main branches length (cm), vine vigor (rate of shoot growth -vine
226 strength, diameter and internode length) - Gruneberg et al. (2010), weight of vines per plot (Kg),
227 number root per plant, number marketable storage roots, number non-marketable storage roots,
228 weight marketable storage roots (Kg), number non-marketable storage roots (Kg), total Root
229 yield (t ha^{-1}), marketable root yield (t ha^{-1}), foliage yield (t ha^{-1}),% of commercial root yield, ratio
230 root length/diameter, root dry matter content (%) and harvest index (HI).

231

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232 **Statistical analysis**

233 The GLM procedure in SAS (ver. 9.1; SAS Institute Inc., Cary, NC) was used for analysis of
234 variance. The two season data were analyzed and are presented separately and combine means
235 for the two seasons. Separation of means was achieved by Tukey's Studentized Range Test. In
236 addition, analysis of variance was used to test for differences between treatments and treatment
237 means were separated by Fisher's protected t-test least significant difference by GenStat (2003).
238 Further, PCA and Pearson correlation coefficients showing pair-wise associations of traits for
239 yield and yield contributing characters was generated by XLSTAT to show the relationship of
240 key parameters measured and treatments.

241

242 **Results**

243 **Symptom expression and virus detection in single or mixed infection by SPFMV, SPCSV**
244 **and SPLCV under field conditions**

245 Analysis of variance for disease severity taken at 90 day after planting showed a significant (*F*
246 *pr.* <.001) interaction between virus treatments for both 'Ejumula' and 'Kakamega' for the two
247 seasons as shown in **Table 2** and **Figure 1.** 'Ejumula' was more severely affected than
248 'Kakamega' for the different virus treatments. Uninfected control treatments for 'Ejumula' and
249 'Kakamega' respectively, did not display symptoms compared to virus-infected treatments.
250 Disease severity scores in both seasons differed among treatments. SPLCV infected plants
251 produced mild symptoms in the two varieties used in this study (**Fig 2A & 3A**). Plants exhibited
252 slight rugosity and upward curling or rolling of leaves. Disease severity due to SPCSV and
253 SPFMV alone was appreciable in both cultivars and both seasons. Uninfected controls were
254 symptomless (**Fig 2B & 3B**). Purple rings characterized the symptom expression due to SPFMV
255 (**Fig 2C & 3C**). SPCSV displayed purpling on older leaves (**Fig 2E & 3E**). A combination of
256 SPCSV+ SPLCV had a more pronounced severity in both seasons on 'Ejumula' - showing
257 chlorotic spots and rugosity; while it was less severe for 'Kakamega'- showing purpling of older
258 leaves and upward curling (**Fig 2D & 3D**). SPVD and SPVD+SPLCV were the most severe for
259 both seasons. These included: vein chlorosis, purple spots, mosaic, leaf narrowing, deformation

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260 and stunted growth (**Fig 2F & 3F**). Worth noting was that symptom severity declined at 120
261 DAP in both seasons. RT-PCR/PCR tests performed from bulk samples at the end of the
262 experiment just before harvest indicated the following plots were contaminated with viruses with
263 which they had not been pre-inoculated: 'Ejumula'- 17 (contaminated by SPLCV), 'Kakamega' -
264 24 (contaminated by SPCSV), Kakamega - 18 (contaminated by SPFMV), in season I and
265 'Kakamega' - 18 (contaminated by SPCSV) in season II (**Table 1**). Because tests were done on
266 bulks, we were unable to determine the extent of contamination, but considering the overall low
267 level of cross-plot contamination observed in bulk testing we assume it was limited to no more
268 than one or two plants/bulk. We could not ascertain the mechanisms of infection for the specific
269 plots, time of infection and if the infection contributed to yield loss. However, as there was no
270 statistically significant difference with the other replicates for the same treatment and symptoms
271 corresponding to those of the contaminating viruses were also not obvious in these plots, we
272 assume they responded to few and late season infections that had minimal impact on plant
273 performance and this was thus not considered during analysis. As no wild *Ipomoea* or
274 sweetpotato fields were present at or near the field trial plots, the source of virus contamination
275 was most likely from adjacent plots.

276

277 **Effects of virus infection on total root yield.**

278 Season I resulted in a greater yield and storage root number (**Supplementary Table 1**) than
279 season II, despite symptoms being generally milder (**Supplementary Table 2**). Significant
280 differences (*F pr.*<.001) were detected, among treatments for root yield related traits (the number
281 of roots per plant, number marketable storage roots, total storage root yield (t ha^{-1}) and ratio root
282 length: diameter) **Table 2**. Analysis demonstrated that total root yield differed significantly for
283 different treatments, variety and season. Uninfected control treatments for 'Ejumula' and
284 'Kakamega' respectively gave a higher storage root yield (t ha^{-1}) compared to the different virus
285 treatment as shown in **Figure 4**. 'Kakamega' infected with SPLCV or all three viruses had a
286 significant yield reduction of 47% and 35% respectively. 'Kakamega' infected with other single
287 or and multiple viruses gave lesser yield reductions ranging from 6% (SPFMV or
288 SPFMV+SPLCV) to 29% (combinations with SPCSV) but were not significant compared to the

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289 control. Contrary, there was no yield reduction for 'Ejumula' infected with SPLCV alone.
290 However, 'Ejumula' infected with all other combinations gave significant yield reduction
291 ranging from 25 – 44 % compared to the uninfected control (**Table 2**). Significant differences (*F*
292 *pr.*<.001) in the ratio root length to diameter, the number of non-marketable roots were observed
293 for some of the different virus treatments. A consistent observation was evident from the virus
294 infected treatments with SPLCV (singly or in combination with SPFMV and or with SPCSV)
295 that produced a high number of fibrous roots compared to the uninfected control treatment
296 (**Supplementary Figure 1**).

297

298 **Yield component traits correlation with the total storage root yield**

299 Yield component traits evaluated for yield and yield contributing characters showed a significant
300 correlation. As observed in **Table 3** total storage root yield (t ha^{-1}) had strong significant positive
301 association with vine vigor (0.654), marketable storage root yield (t ha^{-1}) (0.910), % commercial
302 roots (0.525) and harvest index (0.536). Foliage yield (t ha^{-1}) (0.485) and the number of roots per
303 plant (0.338) recorded a relatively strong but non-significant positive association. Contrary, a
304 negative association was observed between total storage root yield and severity at (- 0.605). In
305 addition, ratio root length to diameter (- 0.387) and the number of non-marketable roots (- 0.337)
306 was negatively correlated with severity, though not significant. The results of the correlation
307 biplot (CB) (**Figure 5)**, supports an association of significant correlated traits for yield and yield
308 contributing characters. Sweetpotato yield parameters varied substantially under the virus
309 treatments, and for both varieties. Total storage root yield (t ha^{-1}), vine vigor, marketable storage
310 root yield (t ha^{-1}), % commercial roots, harvest index, foliage yield (t ha^{-1}) and the number of
311 roots per plant displayed furthest away from the center, were most important to distinguish the
312 virus treatments. The PCA**,** further shows the association of variables (yield parameters) and
313 virus treatments with PCA factor scores in terms of response for the treatments. Uninoculated
314 treatments for 'Ejumula' and 'Kakamega' had the highest storage root yield (t ha^{-1}). Contrary,
315 treatments for 'Ejumula' and 'Kakamega' respectively infected with SPVD+SPLCV were
316 associated with the highest severity scores.

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317

318 **Discussion**

319 Sweepoviruses have increasingly been reported from throughout the world. Despite showing few
320 symptoms; increasingly studies are reporting them to have varying but significant impact on root
321 yields. However, until only very recently (Mulabisana et al., 2019), there had been no reports on
322 their impact on African sweetpotato varieties, and this is the first report of their effect on East-
323 African varieties released from local land-races. We show that SPLCV infected plants produced
324 mild symptoms in both varieties used in this study, which however tended to disappear as plants
325 matured. This expands on earlier investigations that reported lack of any symptoms in SPLCV
326 infected plants (Ling et al. 2010; Lotrakul et al. 2003). Similarly, Mulabisana et al. (2019)
327 recently reported mild to no symptoms depending on varieties infected with two different
328 sweepoviruses whereas Cuellar et al. (2015) demonstrated the effect of virus isolates and plant
329 age on symptom expressions and virus titres. Thus, sweepovirus symptomatology can vary
330 depending on cultivar and virus strain and plant age, but invariably is mild and often absent.

331

332 Results from our trials show that differences in root yield from SPLCV infected sweetpotato
333 were not significantly different from the uninfected treatments for Ejumula for the two seasons.
334 Contrary, Kakamega had a significant yield reduction following single infection with SPLCV.
335 This illustrates varietal differences in response to sweepovirus infection as found by previous
336 studies, reporting yield reductions between 10-94% between different varieties (Clark and Hoy,
337 2006, Ling et al. 2010, Mulabisana et al. 2019). Notable findings compared to previous studies
338 were the clear difference in susceptibility to SPLCV between the two cultivars used in our
339 experiments, where 'Ejumula' which is relatively susceptible to SPFMV and SPCSV appeared
340 insensitive to SPLCV and 'Kakamega' which was more resistant to SPFMV and SPCSV was
341 highly sensitive to SPLCV. This result thus highlights resistance to SPVD and SPLCV (and
342 likely other sweepoviruses) are not necessarily linked. Furthermore, yield losses and symptoms
343 caused by co-infections of SPLCV with SPFMV, SPCSV or both viruses were not significantly
344 different from those caused by the most severe virus in the combination by itself, suggesting a
345 lack of synergistic and limited additive effect of the viruses on yield losses.

346

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347 SPCSV is considered the most damaging virus of sweetpotato due to its ability to induce
348 synergistic viral diseases with several other viruses (Kim et al. 2017), principal and most severe
349 of which is co-infection with SPFMV, causing SPVD. By itself SPCSV may cause mild to
350 severe symptoms of yellowing or reddening of older leaves, which can often be confused with
351 nutritional deficiencies (Untiveros et al. 2007). Corresponding to the level of resistance of the
352 varieties, single infection by SPCSV induced pronounced symptoms in 'Ejumula' (considered
353 susceptible) and produced milder symptoms in 'Kakamega' (considered tolerant) and led to yield
354 losses of 38% and 24% on average over both seasons respectively. Co-infection with SPFMV
355 increased symptom severity and yield loss in both cultivars. In 'Ejumula' yield losses were
356 significantly different between plants infected by SPFMV, SPCSV or both viruses, whereas in
357 'Kakamega' the yield los between SPCSV, SPFMV, and co-infected plants was identical. This
358 contrasts with most previous reports where more severe yield reductions (from 60-95%) were
359 found when plants co-infected by SPCSV and SPFMV (Milgram et al. 1996; Gibson 1998 and
360 Gutierrez et al. 2003) and may be a result of the specific virus strains and/or varieties used in the
361 current experiment. Further infection of SPLCV in combination with SPFMV and SPCSV, led to
362 slightly higher (non-significant) yield reductions compared to SPFMV and SPCSV alone in both
363 cultivars.

364 Gutierrez et al. (2003) and Tugume et al. (2013), reported that SPFMV-infected Jonathan and
365 Constanero varieties did not show foliage symptoms under field conditions. Nevertheless, typical
366 symptoms associated with SPFMV were observed in the current study and also by Mulabisana et
367 al. (2019). However, as with SPLCV, a reduction of symptoms was observed in SPFMV
368 infected plants as they matured and most plants became symptomless after 16 weeks. This
369 phenomenon has been reviewed by Gibson and Kreuze (2015) who reported that popular East
370 African cultivars appear to sustain their long-term survival by reverting to symptomless infection
371 and even becoming virus free in some occasions. In our trials, SPFMV by itself had a significant
372 yield impact on 'Ejumula', whereas 'Kakamega' was not affected, which is in concordance with
373 their level of resistance. Previous investigations have also presented contradictory conclusions
374 regarding yield reductions by single SPFMV infections. Milgram et al. (1996) and, Clark and
375 Hoy (2006) and Gutierrez et al. (2003), noted that single infection with SPFMV did not greatly
376 affect yield. On the other hand, yield reduction of up to 46% were reported in other studies
377 (Gibson et al. 1997; Mukasa 2004; Njeru et al. 2004 and Domola et al. 2008), and recently,

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378 Mulabisana et al. (2019) reported reductions of 27-92% by single infection with SPFMV across
379 12 different cultivars in field trials in South Africa with notable between season effects. Thus, as
380 in the case of sweepoviruses and SPCSV, the impact of SPFMV single infection on yield seem to
381 be highly cultivar specific and across all cultivars globally may be higher than previously
382 assumed. Gibson and Kreuze (2015), have comprehensively documented previous work on yield
383 reductions reported by treatments and cultivars.

384 Previous investigations have documented that yield and quality of storage roots are sensitive to
385 environmental variations: from year to year, field to field, and even within the same field
386 (Collins et al. 1987; Ngeve and Bouwkamp, 1993 and Bryan et al. 2003). Yield variation among
387 treatments and seasons could be attributed to climatic factors like rainfall and temperature
388 (Roitsch et al. 2003). In season II, we experienced a fourfold increase in rainfall compared to
389 season I (**Supplementary Figure 2**), but no significant difference in temperatures. Sweetpotato
390 is sensitive to water logging and too much water, specifically early in the growing season, could
391 have led to a lower yield than the first season.

392

393 Principal component analysis (PCA) biplot, supports an association of significant correlated
394 traits for yield and help identify yield contributing characters. Furthermore, it shows the
395 relationship of variables (yield and yield contributing traits) and observation (virus treatments)
396 scores, with PCA factor scores in terms of response for the treatments. For instance,
397 uninoculated treatments for var. Ejumula and Kakamega had the highest storage root yield (t ha^{-}
398 ^{1}). Contrary, treatments inoculated with SPVD+SPLCV for 'Ejumula' and 'Kakamega' were
399 associated with the highest severity scores and low root yield. Understanding interrelationships
400 among various yield and yield contributing characters is important and can be utilized by
401 breeders when evaluating for virus tolerant varieties during selection.

402 The highest negative and significant association existed between total storage root yield and
403 disease severity, in both varieties. Gurmu et al. (2015), described a negative correlation between
404 virus symptoms and root yield and is consistent with present results. SPVD is a damaging
405 disease complex of sweetpotato and the negative correlation observed between fresh root yield
406 and disease severity was expected. In addition, ratio root length to diameter, the number of non-

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407 marketable roots were negatively corelated to root yield, though not significant. These findings
408 corroborate Bryan et al. (2003) who noted that virus infected planting material produced storage
409 roots with a high length/diameter ratio, culminating in lower total yield and root quality.

410

411 **Conclusions**

412 Our study confirmed the relative susceptibility to SPVD of 'Ejumula', and revealed it expressed
413 equal sensitivity to both viruses. The relatively SPVD tolerant phenotype of 'Kakamega', was
414 expressed as reduced symptoms and absence of yield penalties upon SPFMV infection and
415 reduced symptoms and yield losses upon SPCSV infection as compared to 'Ejumula'. On the
416 other hand, in contrast to other studies and despite the obvious enhancement of symptoms in
417 SPVD affected plants of both cultivars, we found no evidence of synergistic yield reductions as
418 compared to single infections and suggests that symptoms may not always be an adequate
419 indicator for the effect on yield. This was also clearly the case for SPLCV infection. Considering
420 the widespread presence of begomoviruses globally and also in Africa, this suggests that
421 breeders need to take into account these viruses when selecting for SPVD resistance, as they may
422 inadvertently be selecting for sweepovirus susceptibility by ignoring them. Nevertheless, even in
423 'Kakamega', SPLCV infections induced only mild symptoms that disappeared over time, making
424 such plants difficult to identify to farmers, seed producers and breeders alike to implement any
425 control methods. Thus, adequate diagnostic tests are needed to support these efforts. No effective
426 antisera are available for sweepoviruses and the PCR tests used in this study are too cumbersome
427 for routine implementation in breeding programs or seed certification systems. An effort into
428 developing easier to use molecular diagnostics for sweepoviruses based on isothermal
429 amplifications systems is recommended to support these efforts.

430 On the other hand, although only one sweepovirus isolate was used in this study, we know from
431 previous studies that this group of viruses is hugely variable and that different isolates differ in
432 their ability to provoke symptoms in sweetpotatoes and indicator plants and accumulate at
433 different titres (Cuellar et al., 2015). Important questions that remain to be answered are if
434 different isolates/species differ in their impact on sweetpotato root yield, if this can be correlated
435 to any particular characteristics other than symptoms (such as virus titres) and if resistance of

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436 sweetpotatoes to one of them is correlated with resistance to other isolates. Thus, immediately
437 relevant research topics include evaluating the extent of sweepovirus infections as well as the
438 virus variability in farmers fields in Kenya and Africa in general and the susceptibility to these
439 viruses of current sweetpotato varieties, particularly those selected for resistance to the more
440 visible SPVD.

441 **Competing interests**

442 The authors declare that they have no competing interests.

443

444 **Author Contributions**

445

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454 **Writing - review & editing:** Bramwel W. Wanjala, Elijah M. Ateka, Jan F. Kreuze, Douglas W.
455 Miano and Jan W. Low. All authors read and approved the final manuscript.
456

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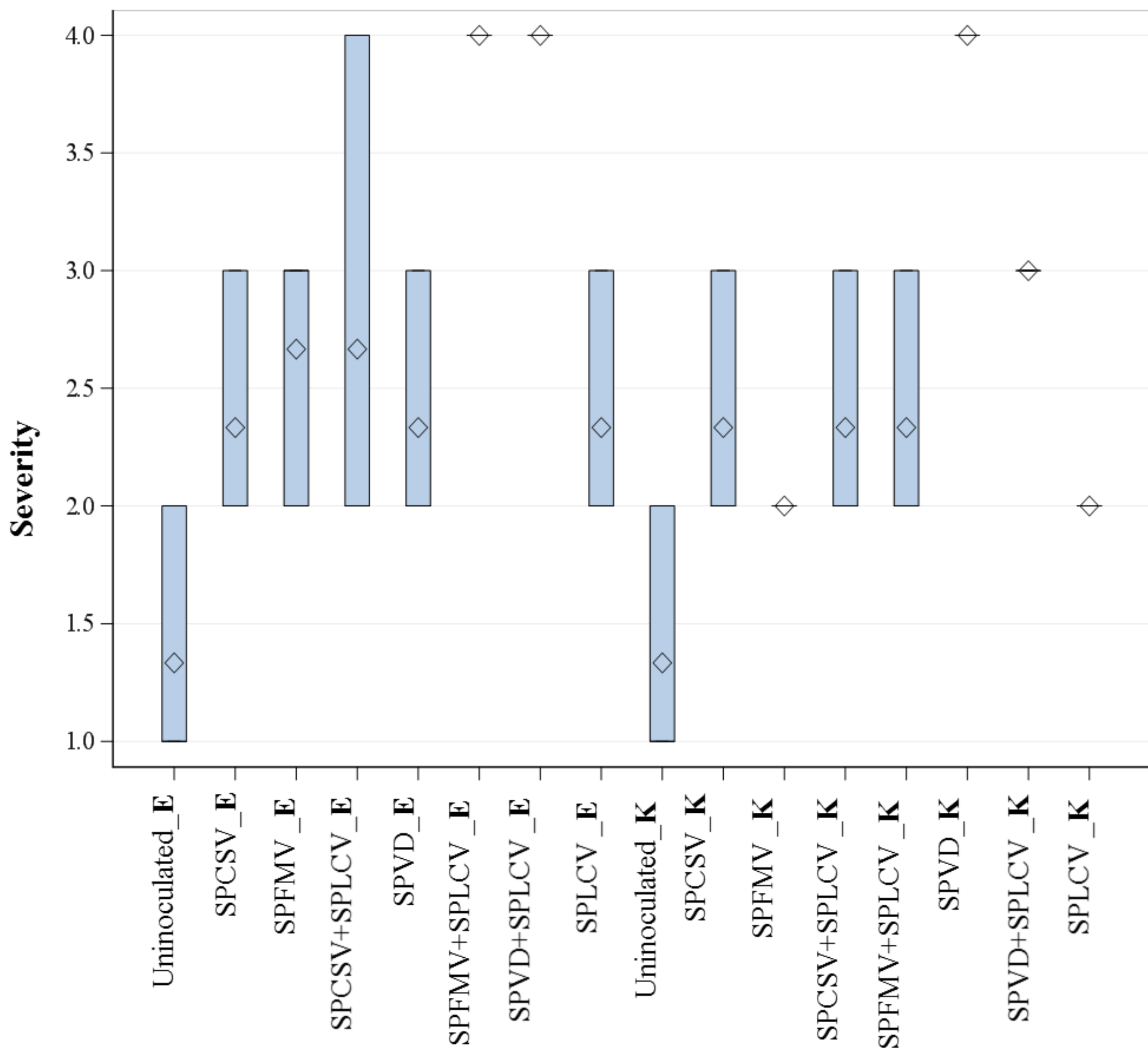

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675 **Figure 1**: Box-plot for disease severity inoculated with different viruses for combined means for
676 season I_II; expressed varying level of disease symptoms. Severity score of 1 depicts mild
677 symptom expression while 5 is pronounced. All the treatments for Ejumula are abbreviated **E**
678 and **K** for Kakamega.

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684 **Figure 2:** Symptom expression due to single or mixed infection on 'Ejumula' by *Sweet potato*
685 *leaf curl virus* (SPLCV), Sweet *potato feathery mottle virus* (SPFMV), and *Sweet potato*
686 *chlorotic stunt virus* (SPCSV) under field conditions. A – rugosity due to SPLCV, **B** –
687 uninfected, **C -** purple spot due to SPFMV, **D -** rugosity and chlorotic spots due to
688 SPCSV+Begomo, **E -** purpling of older leaves due to SPCSV and **F -** vein clearing, chlorosis,
689 leaf reduction/deformation–SPVD.

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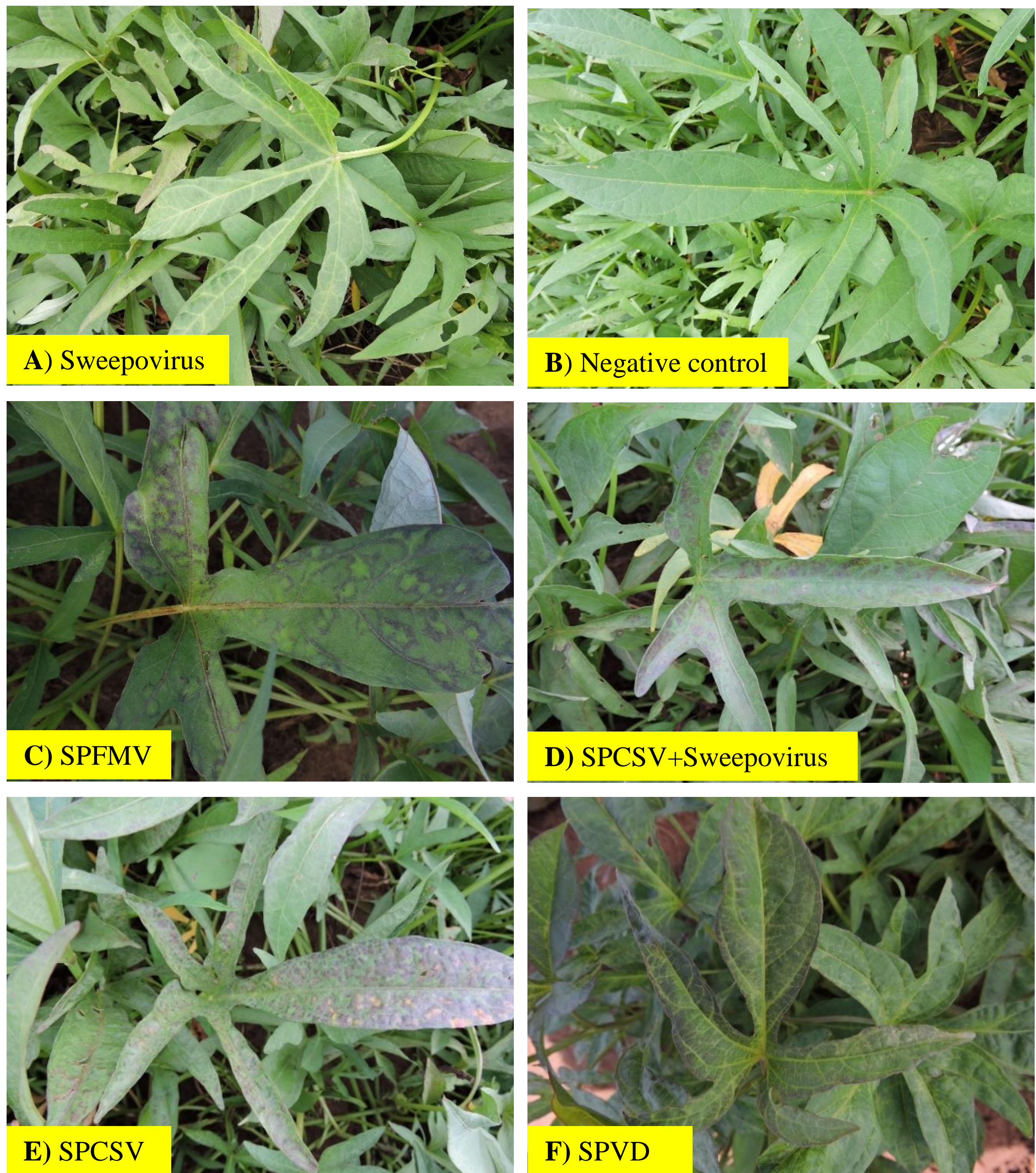

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692 **Figure 3:** Symptom expression due to single or mixed infection on 'Kakamega' by *Sweet potato*
693 *leaf curl virus* (SPLCV), Sweet *potato feathery mottle virus* (SPFMV), and *Sweet potato*
694 *chlorotic stunt virus* (SPCSV) under field conditions. A – chlorosis and vein clearing to SPLCV,
695 **B** – uninfected, **C -** purple spot due to SPFMV, **D -** purpling and roll up due to SPCSV+Begomo,
696 **E -** bottom left – purpling of older leaves due to SPCSV and **F -** vein clearing, chlorosis, leaf
697 reduction/deformation–SPVD.

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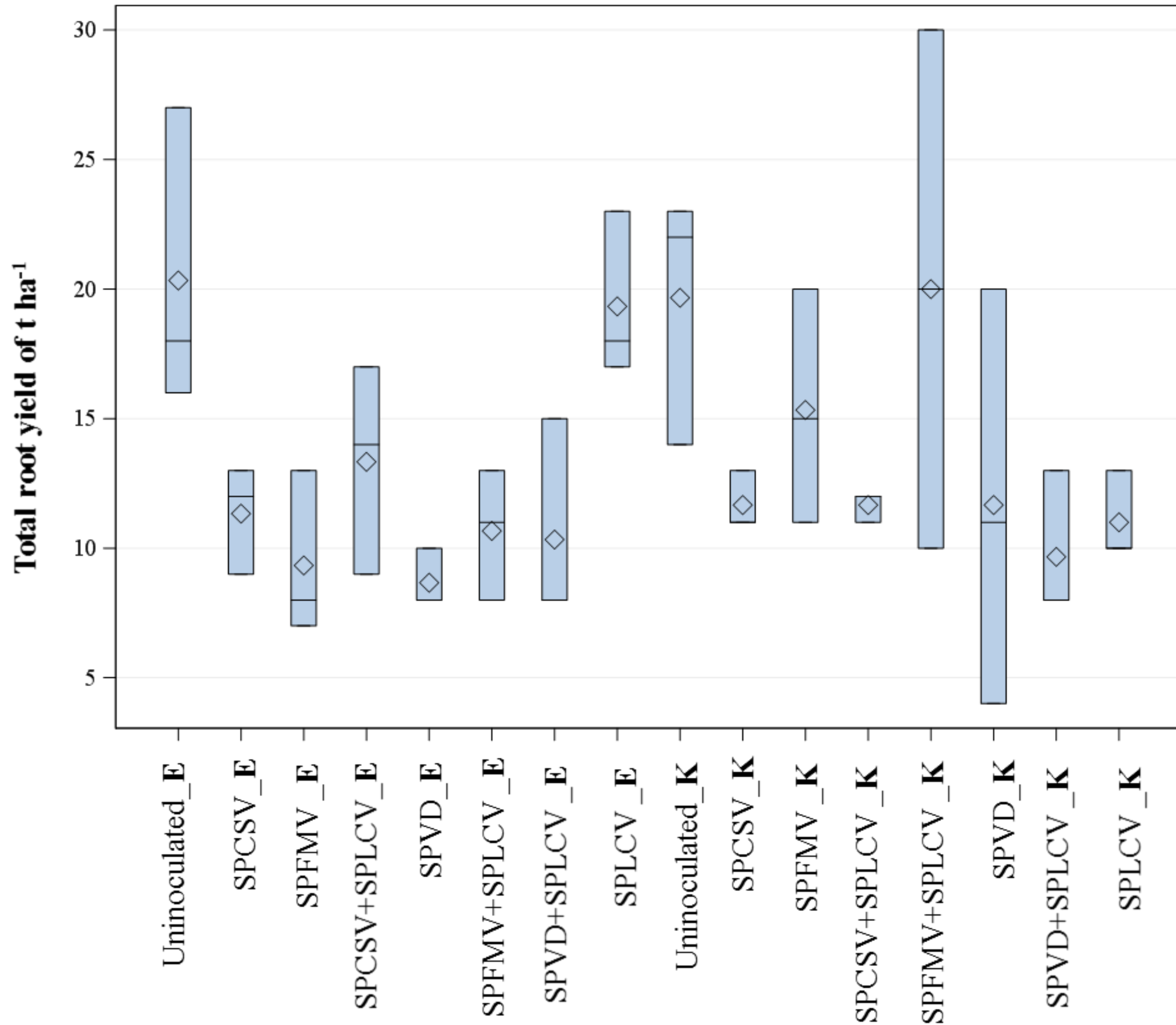
699 **Distribution of total root yield of t ha^{-1}**



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701 **Figure 4**: Box-plot root yield in t ha^{-1} for treatments inoculated with different viruses. All the
702 treatments for Ejumula are abbreviated **E** and **K** for Kakamega.

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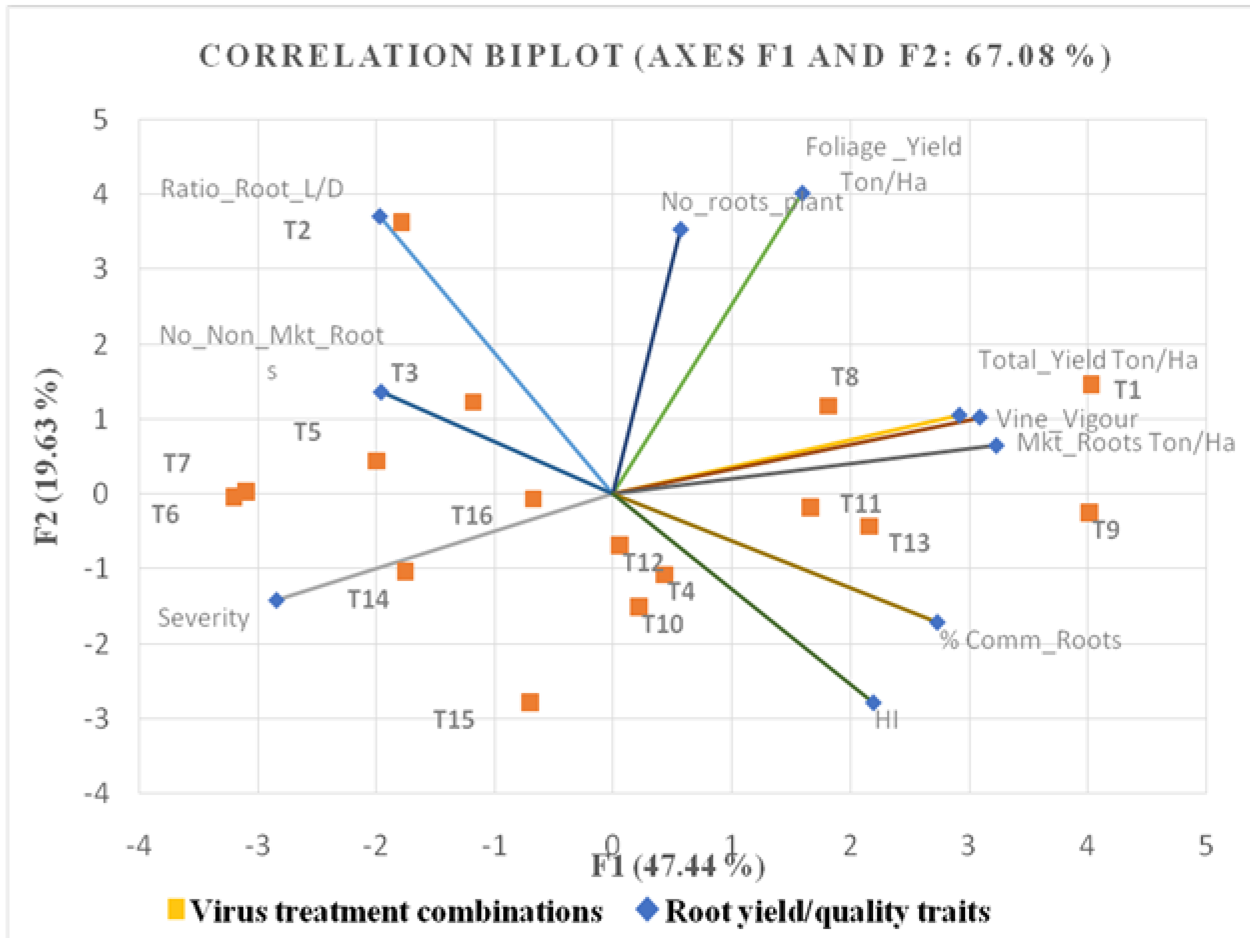

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710 **Figure 5**: Correlation biplot (CB) representing root yield/quality traits observations and virus
711 treatment variables. Narrow angles depict positively related observations, right angle unrelated
712 and obtuse (wide) angle negatively related. See **Table 1** for treatment descriptions.

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745 **Supplementary Figure 1**: Effect of SPLCV+SPCSV on root formation of Var. Kakamega; A_1
746 – vigorous above ground cover and A_2 – fibrous root formation and B_1 and B_2 – vigorous
747 ground cover and B_2 – good root formation.

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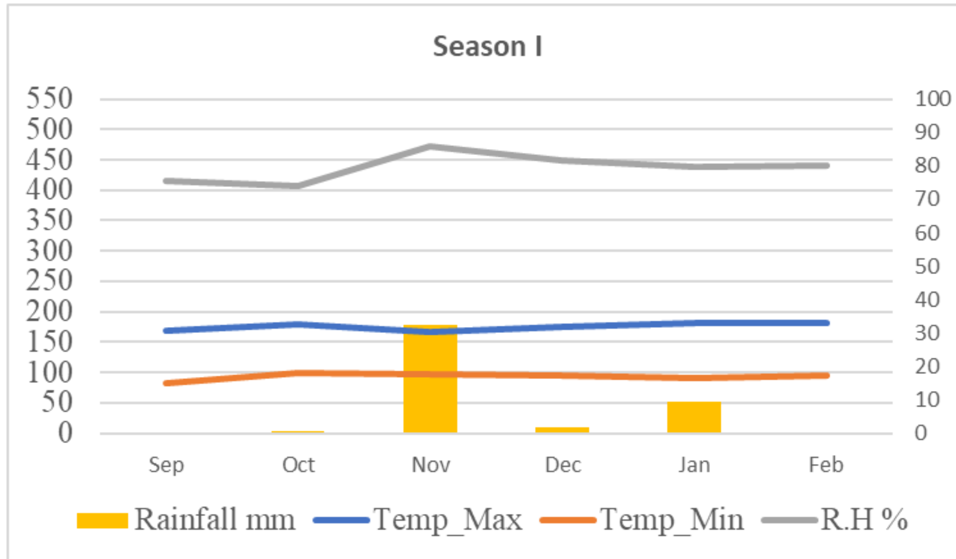

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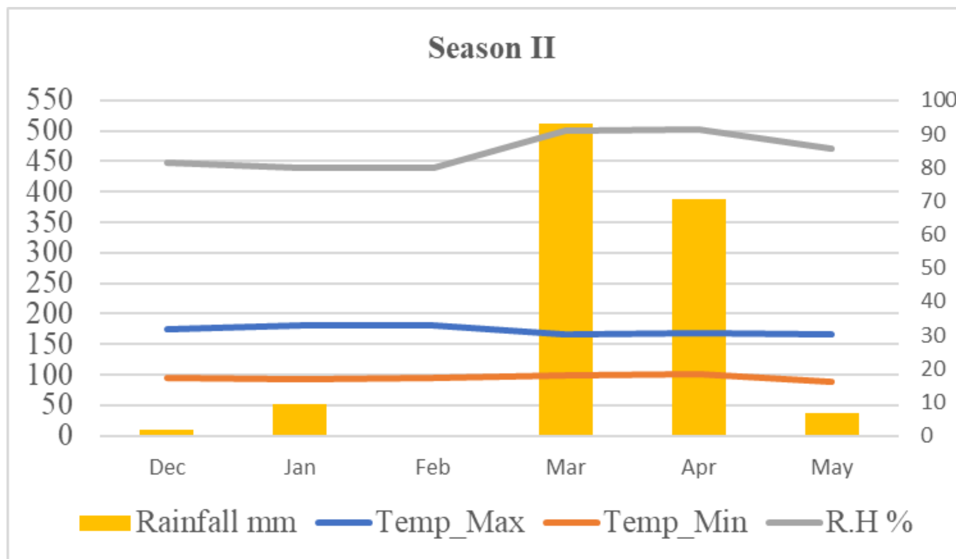

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753 **Supplementary Figure 2.** Monthly average climate data (2017 - 2018) rainfall (left axis),
754 temperature, relative humidity (right axis) at KALRO Kiboko, Makueni, Kenya.
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756 Data were made available courtesy ICRISAT field station Kiboko.

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759 **Table 1**. Description of treatments (viruses and their combinations) used to evaluate the effect of
760 different viruses on sweetpotato varieties Ejumula and Kakamega.

Treatment	Plot No	Cultivar	Treatment description
T1	15/30/43		Non infected
T2	5/17[1]/40		SPCSV
T3	16/31/47		SPFMV
T4	9/27/41	Ejumula	SPLCV +SPCSV
T5	3/22/33		SPLCV +SPFMV
T6	12/23/46		SPVD
T7	14/19/36		SPLCV +SPVD
T8	10/25/44		SPLCV
T9	4/24[2]/39		Non infected
T10	11/29/34		SPCSV
T11	1/20/48		SPFMV
T12	7/21/35	Kakamega	SPLCV +SPCSV
T13	6/32/42		SPLCV +SPFMV
T14	8/26/38		SPVD
T15	13/28/37		SPLCV +SPVD
T16	2/18[3]/45		SPLCV

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762 **Key**: SPCSV - *Sweet potato chlorotic stunt virus*, SPFMV - *Sweet potato feathery mottle virus* ,
763 SPLCV – *Sweet potato leaf curl* virus and SPVD - *Sweet potato virus disease.*

764 [1] Plot positive for sweepovirus in bulk PCR test at end of season I

765 [2] Plot positive for SPCSV in bulk PCR at end of season I

766 [3] Plot positive for SPFMV in bulk PCR test at end of season I, and SPCSV at end of season II

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769 **Table 2**. Yield parameters for sweetpotato inoculated with *Sweet potato leaf curl* virus (SPLCV), *Sweet potato feathery mottle virus*
770 (SPFMV), and *Sweet potato chlorotic stunt virus* (SPCSV), alone and in all possible combinations on varieties Ejumula and
771 Kakamega for Season I and Season II combined; Values shown are means for the 16 treatments.

	Yield parameter Season I and II					
Treatment	Severity (1-5)	Number roots per plant	Number marketable storage roots	Ratio root length: diameter	Total root yield t ha^{-1}	% Yield reduction
Negative Cntl_Ejumula	1.33 e	6.00 abcd	29.33 ns	3.573 bcd	15.92 abc	Control
SPCSV_Ejumula	2.33 cd	4.82 efgh	24.17 ns	4.81 a	10.11 e	38
SPFMV_Ejumula	2.67 bc	5.02 defgh	25.17 ns	3.84 b	9.14 e	44
SPLCV+SPCSV_Ejumula	2.67 bc	5.28 bcdef	21.00 ns	3.365 bcd	11.56 bcde	25
Sweepo +SPFMV_Ejumula	2.17 cd	5.25 bcdef	22.67 ns	3.816 bc	9.17 e	44
SPVD_Ejumula	3.67 a	4.43 fgh	22.17 ns	3.633 bcd	11.25 cde	32
SPLCV +SPVD_Ejumula	3.67 a	4.03 h	21.17 ns	3.664 bcd	9.11 e	44
SPLCV _Ejumula	2.17 cd	5.0.7 cdefg	26.50 ns	3.369 bcd	15.64 abcd	0
Negative Cntl_Kakamega	1.17 e	6.17 ab	22.83 ns	3.225 d	16.50 a	Control
SPCSV_Kakamega	2.33 cd	5.63 abcde	19.33 ns	3.428 bcd	13.28 abcde	24
SPFMV_Kakamega	2.00 d	6.15 ab	22.67 ns	3.856 b	15.78 abcd	6
SPLCV +SPCSV_Kakamega	2.17 cd	5.75 abcde	19.17 ns	3.108 d	12.22 abcde	29
SPLCV +SPFMV_Kakamega	2.17 cd	6.00 abcd	19.50 ns	3.525 bcd	16.17 ab	6
SPVD_Kakamega	3.83 a	6.07 abc	13.67 ns	3.86 b	12.75 abcde	24
SPLCV+SPVD_Kakamega	3.00 b	6.57 a	16.83 ns	3.247 cd	10.94 de	35
SPLCV _Kakamega	2.00 d	4.227 gh	17.33 ns	3.585 bcd	9.08 e	47
F pr.	<.001	<.001	0.053	<.001	<.001	
LSD 5 %	0.538	*1*	*8.161*	*0.575*	*4.9*	
cv%	8.1	5.8	8.2	1.6	9	

772 * Values within the same column followed by identical letters are not statistically different (ns – not significant). ANOVA, Fisher's
773 protected Least Significance Difference test.
774

775 **Table 3**. Pearson correlation coefficients showing pair-wise associations for yield and yield contributing characters of sweetpotato var.
776 Ejumula and var. Kakamega using virus tested and virus infected (with SPLCV, SPFMV, and SPCSV, alone and in all possible dual
777 combinations) evaluated for two seasons in Kenya.
778

Variables	**Severity**	**Vine Vigor**	**Ratio Root Length/ Diameter**	**Foliage Yield Ton/Ha**	**No. roots per plant**	**Total root yield t ha^{-1}**	**Mkt Roots Ton/Ha**	**% Comm Roots**	**No. Non Mkt Roots**	**Harvest index (HI)**
Severity	**1**									
Vine_Vigour	**-0.925**	**1**								
Ratio_Root_L_D	0.204	-0.189	**1**							
Foliage _Yield Ton/Ha	-0.485	0.364	0.180	**1**						
No_roots_plant	-0.137	0.192	0.188	0.319	**1**					
Total_Yield Ton/Ha	**-0.605**	**0.654**	-0.387	0.485	0.338	**1**				
Mkt_Roots Ton/Ha	**-0.599**	**0.605**	-0.482	**0.547**	0.283	**0.910**	**1**			
% Comm_Roots	**-0.590**	0.490	**-0.653**	0.285	-0.283	**0.528**	**0.678**	**1**		
No_Non_Mkt_Roots	0.296	-0.428	0.371	-0.193	0.153	-0.337	-0.420	-0.426	**1**	
Harvest index (HI)	-0.313	0.494	**-0.629**	-0.325	0.069	**0.536**	**0.528**	0.405	-0.333	**1**

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780 Values in bold are different from 0 with a significance level alpha = 0.05
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