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Performance of *Solanum aethiopicum* Shum group accessions under repetitive drought stress

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Drought is a serious climatic hazard to crop production, more especially when it occurs repeatedly. This created a need to identify repetitive drought tolerant varieties that recover following exposure to drought. Twenty accessions of *Solanum aethiopicum* Shum group were evaluated for their response to repeated drought exposure in a screen house at Uganda Christian University stressed and well-watered conditions in a split-plot arrangement. Data was collected on growth and yield parameters namely leaf area, plant canopy width, plant height, plant branching, fresh leaf weight, fresh shoot biomass, and harvest index. Exposure of plants to repetitive drought stress led to significant decrease in all evaluated growth parameters at p<0.001 except for plant branching. Similarly, yield parameters exhibited a highly significant difference among accessions and between water levels at p<0.001. Principal component analysis of growth rate traits showed that leaf area contributed to the highest variation for recovery from repetitive drought stress among accessions. The accessions that recovered best from drought stress include SAS108/2015, SAS163/P/2015, SAS183/G/2015, and SAS168/G/2015. For yield parameters, the accessions SAS137/2015, SAS148/2015, SAS108/P/2015, and SAS160/2015 had the highest dry shoot biomass. These findings indicate prospect for improvement of tolerance to repetitive drought stress in *S. aethiopicum* Shum group.

Key words: African eggplant, leafy vegetable, drought tolerance, principal component analysis, growth response.

INTRODUCTION

Solanum aethiopicum Shum (African eggplant) is an African indigenous vegetable crop and majorly produced in peri-urban areas of sub-Saharan Africa (Lim, 2013). It is grown for its edible leaves (Adeniji et al., 2013) which are rich in nutrients like proteins, vitamin A, Fe and Ca that help to boost the body' immune system (Sodamade et al., 2015). The *S. aethiopicum* Shum group thrives under warm humid conditions although its leaf quality

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quality and yield are affected by limited water supply during growth. According to Kumar et al. (2012), leafy vegetables are more susceptible to moisture deficit stress than other crops owing to wilting effects that reduce market value. More so, repetitive drought episodes within a cropping cycle are common as one of the major aspects of climate change but the associated crop performance effects in the *S*. *aethiopicum* had not been investigated (Mwaura and Okoboi, 2014). The drought periods recurring in an irregular manner can significantly affect the crop physiological and morphological appearance; hence, leading to a reduction in both growth rate and yield (Kumar, 2013). Agricultural regions affected by drought can experience yield losses of over 50% (Bahadur et al., 2011). The low yields of green leafy vegetables due to drought exposes many people to the danger of food and nutrition insecurity (Lotter et al., 2014). For that reason, vegetables are usually grown during rainy seasons or where there is a reliable source of water for production. In dry seasons, farmers usually grow their vegetables only near wetlands or swamps (Lim, 2013). Nonetheless, *S*. *aethiopicum* is an affordable leafy vegetable whose increased production and consumption could lead to dietary improvement (Pincus, 2015).

Among the morphological traits affected by drought, shoot and leaf growth is often more inhibited compared to root growth. This occurs as a result of impaired mitosis of cell thereby reducing plant height, leaf area and crop growth (Sudarmonowat et al., 2012). There is insufficient knowledge on the performance of *S*. *aethiopicum* Shum group under repetitive drought stress. This is compounded by the lack of information on availability of drought tolerant germplasm, making it difficult to develop new varieties. The main objective of this study was to identify *S*. *aethiopicum* accessions that could recover from repetitive drought stress. Specifically, the objectives of the study were to determine the effect of repetitive drought stress on (i) growth rate and (ii) yield of *S*. *aethiopicum* Shum group accessions. Knowing repetitive drought resistant accessions could guide breeders in developing climate smart varieties (Schafleitner et al., 2015).

MATERIALS AND METHODS

Study location and experimental materials

The study was conducted at Uganda Christian University, Mukono in the screen house. Mukono is located in the central part of the country approximately 27 km East of Kampala. It lies at an altitude of 1158 to 1219 m above sea level. Mukono receives two wet seasons with an annual rainfall ranging between 1100 and 1400 mm. The temperature ranges between 21 and 29°C with coordinates 00°20'N 32°45'E. The experiment was carried out between November 2016 and April 2017, during which the range day screen house temperature and relative humidity were 18.1 to 51.5°C and 28.5 to 87%, respectively. Twenty *S. aethiopicum* Shum group accessions (Table 1) used in this study were obtained from seed bank at the Department of Agricultural and Biological Sciences, Uganda Christian University. The study accessions differed morpho-agronomically (Sseremba et al., 2017).

Experimental design

A factorial experiment composed of accessions and moisture deficit stress was laid in a split-plot arrangement with 2 replications. The main plot factor was moisture deficit stress with 2 levels namely, stressed (25% field capacity, FC) and well-watered (100% FC). An earlier study by Kesiime (2016) showed that the 25% FC imposes sufficient drought stress to enable cultivar discrimination in a related species, *S*. *tuberosum*. The sub-plot factor was the accessions (genotypes) with 20 levels. Each sub-plot consisted of 3 potted plants of a genotype.

Determining field capacity

The FC was estimated as earlier applied by Kesiime et al. (2016) and it is briefly described here. A sample of soil was put in the weighed container (W1) and weight of the soil sample plus the container was gotten (W2) using a weighing balance. The soil sample was oven dried for 24 h at a temperature of 105°C (Govet et al., 2010) followed by re-weighing (weight of the dried soil plus the container, W3). The dried soil sample was poured in the sieve and a known quantity of water (Wt_1) left to run through. The experiment was left to stand until the water has completely drained from the soil. Seeped-out water was recorded using a measuring cylinder (Wt_2). The FC was then calculated as follows; $= Wt1 - Wt2$. The estimated FC was used to calculate the amount of water to use in the pots at particular intervals (to apply the repetitive drought stress). For 10 kg of potting substrate, the 100% FC was estimated to be 2.4 L, while 25% was estimated 0.6 L.

Preparing potting substrate

A potting substrate composed of a mixture of topsoil and well decomposed cow dung manure in a ratio (3 soil:1 manure). Before its potting, the substrate was steam-sterilized to kill off any pathogenic organisms and weed seeds. Each pot (polythene type) was filled with 10 kg of the soil mixture. The uniformly filled pots were then arranged according to the split-plot design. The pots were spaced 45 and 15 cm between and within sub-plots, respectively.

Raising seedlings and transplanting

One hundred seeds per accession were sown in well-labeled and large pots for raising seedlings in the screen house. In the nursery, routine management practices were carried out to ensure proper growth. Transplanting seedlings to pots was carried out at 4 weeks after sowing (4 to 5 leaf stage). During transplanting, seedlings with uniform growth per accession were selected. Potted plants were provided with 4 g of fertilizer N.P.K (17:17:17) per pot on a fortnightly basis. Spraying against insect pests and mites was done every 2 weeks using dimethoate and cypermethrin.

Applying repetitive drought stress

Watering of the transplanted seedlings was done on a daily basis until one week after transplanting (WAT) to ensure proper seedling establishment. At the end of the first WAT, the *ad libitum* (unrestricted) watering was stopped for the next one week, at the

Table 1. The different accessions used in the study.

Accession code	Name	Accession code	Name
G1	SAS168/G/2015	G11	SAS148/2015
G2	SAS183/G/2015	G12	SAS145/2015
G3	SAS163/2015	G13	SAS168/P/2015
G4	SAS163/P/2015	G14	SAS184/G/2015
G5	SAS157/P/2015	G15	SAS137/2015
G6	SAS160/2015	G16	SAS184/P/2015
G7	SAS163/G/2015	G17	SAS141/2015
G8	SAS183/P/2015	G18	SAS108/P/2015
G9	SAS108/2015	G19	SAS185/G/2015
G10	SAS157/G/2015	G20	SAS185/P/2015

Extracted and modified from Sseremba et al. (2017).

end of which the 2 water deficit stress treatments namely well-watered or 100% FC (2.4 L/pot) and stressed or 25% FC (0.6 L/pot) were introduced. From 2 WAT, the watering was carried out once every week at specific drought stress levels. The repeated 7-day intervals among watering treatment points constituted the repetitive drought stress.

Data collection

Effect of repetitive drought stress on growth rate

Data on growth parameters was collected before re-watering at the end of 3rd, 5th and 7th WAT. The morphological attributes for growth rate that were evaluated include leaf length (cm) and leaf width (cm) to obtain leaf area (cm^2) by multiplying the length by width, number of leaves per plant, plant height (cm), plant branching (number of branches per plant) and plant canopy width (cm). The observational unit was an individual plant in a polythene pot.

Effect of repetitive drought on yield

At 7 WAT, the following yield traits were measured, namely, leaf weight (g) and shoot weight (g). In addition, harvest index (HI) was calculated. During the harvesting, the weight measurements were immediately taken (before getting scotched) using a highly sensitive weighing balance (Mettler Teledo, EL 303 Max 320 g, d = 0.001 g). For leaf weight (fresh weight), 5 most fully open leaves (N=5) from top of the plant were picked at 7 WAT, weighed and mean values recorded per plant. The shoot was cut from the soil level of a pot at harvest at 7 WAT and immediately weighed together with corresponding leaves that had already been removed for leaf weights. The harvest index (HI) was obtained using the following formula:

$$\mathrm{HI} = \frac{\mathrm{LPP} \times \mathrm{LW}}{\mathrm{SW}}$$

where LPP represents the number of leaves per plant, LW represents the leaf weight and SW represents the shoot weight.

Data analysis

An analysis of variance (ANOVA) was performed in GenStat statistical computer package (12th edition, version 2; VSN International Ltd, 2009) to examine variations among accessions. The following split-plot linear models were analyzed: (i) Split-plot design [replications (blocks) as a factor]: $y_{ijk} = \mu + R_i + W_j + G_k + \varepsilon_{ijk}$ and (ii) Split-plot design (blocks not a factor); $y_{ijk} = \mu + +W_j + G_k + \varepsilon_{jk}$; where y is the variable recorded, μ is the overall mean, R stands for replications, W is the water deficit level, G refers to access ions and ε the random error. The replication was considered as a random factor, and in both models, the water deficit stress and accession were considered as fixed factors. Mean values of accession performance for different traits at each moisture deficit level were computed. Principal component analysis (PCA) was also carried out in GenStat on traits where significant variation (at α = 5%) was observed from the ANOVA (F-test) to identify parameters that account for most of variation among the study accessions.

RESULTS

Effect of repetitive drought stress on growth rate

Blocking had no significant effect on growth parameters and results from the first model that had considered blocking as a factor are not presented. Based on results of the second model (blocks not a factor), there was a significant difference between the stressed and well-watered plants ($p<0.05$) for all the measured growth parameters, except for plant branching. At least, 2 accessions were also significantly different for each of the growth parameters measured within drought stressed ($p<0.05$) and well-watered ($p<0.05$) plants.

Three weeks after transplanting (3 WAT)

The control (100% FC) showed a mean; leaf area of 98.91 cm^2, number of leaves 17, plant height of 7.89 cm, plant canopy of 25.12 cm and plant branching of 6. The stressed plants showed a mean; leaf area of 34.83 cm, number of leaves of 11, plant height of 5.40 cm, plant canopy of 20.84 cm and plant branching of 4.The mean difference in leaf area was 34.82 ± 7.49 cm^2, number of leaves was 6 ± 2, plant height was 2 .49 ± 1.38 cm, plant

Table 2. The first four components based on principal component analysis.

Growth traits	PC1	PC2	PC3	PC4
% variation	80.230	7.740	5.170	3.390
LA_3WAT	-0.202	0.667	0.653	0.217
LA_5WAT	-0.594	0.471	-0.646	0.010
LA_7WAT	-0.777	-0.531	0.317	-0.097
LPP_3WAT	0.008	0.058	0.011	-0.208
LPP_5WAT	0.013	0.070	0.009	-0.379
LPP_7WAT	0.038	0.134	0.001	-0.759
PB_3WAT	0.002	0.011	0.007	-0.043
PB_5WAT	0.004	0.017	0.006	-0.049
PB_7WAT	0.005	0.013	0.006	-0.058
PH_3WAT	0.001	0.032	0.046	-0.067
PH_5WAT	-0.005	0.059	0.105	-0.225
PH_7WAT	-0.025	0.122	0.202	-0.343
PW_3WAT	-0.017	0.075	0.035	-0.025
PW_7WAT	-0.030	0.004	-0.022	-0.009

canopy was 4.28 ± 0.11 cm and plant branching was 1 ± 0.3 (control minus 25% FC). Generally, repetitive drought had most effect on the evaluated traits at 25% FC except for plant branching. However, there was a significant difference among accessions and the different water levels ($p<0.05$).

Five weeks after transplanting (5 WAT)

The control (100% FC) showed a mean; leaf area of 179.35 cm^2, number of leaves of 30, plant height of 20.42 cm, plant canopy of 30.81 cm and plant branching of 9. The stressed plants showed a mean; leaf area of 69.92, number of leaves of 20, plant height of 12.72, plant canopy of 26.35 cm and plant branching of 7. The mean difference in leaf area was 69.92 ± 24.11 cm^2, number of leaves was 10 ± 2, plant height was 7.71 ± 2.31 cm, plant canopy was 4.47 ± 0.5 cm and plant branching was 1 ± 0.1 (control minus 25% FC). Generally, repetitive drought had its most effect on all traits at 25% FC except for plant branching that was not affected. However, there was a significant difference among accessions and the different water levels.

Seven weeks after transplanting (7 WAT)

The control (100% FC) showed a mean; leaf area of 209.56 cm^2, number of leaves of 47, plant height of 37.54 cm, plant canopy of 34.83 cm and plant branching of 11. The stressed plants showed a mean; leaf area of 77.91, number of leaves of 31, plant height of 21.99 cm, plant canopy of 30.34 cm and plant branching of 10. The mean difference in leaf area was 77.91 ± 32.9 cm^2, number of leaves was 17 ± 4, plant canopy was 4.49 ± 0.52 cm, plant height was 15.54 ± 2.40 cm and plant branching was 1 ± 0.2 (control minus 25% FC). Generally, repetitive drought had most effect on all evaluated traits except for plant branching at 25% FC. However, there was a significant difference among accessions and the different water levels.

Principal Component Analysis (PCA) of growth parameters

A PCA was used to identify traits that can distinguish among accessions under repetitive drought stress. From Table 2, the first PCA produced the highest loadings for leaf area (LA); LA at 7 WAT was leading (loading -0.777) followed by LA at 5 WAT (loading -0.594) and LA at 3 WAT (loading -0.202).

Based on the obtained results from the separation of means and the PCA, leaf area was used as a basis for selection of repetitive drought stress tolerant accessions. An average of the leaf area at different stages (3 WAT, 5 WAT and 7 WAT) was gotten for both control and stressed plants. The average difference between the control and the stressed plants was calculated. Accessions with the smallest difference in performance under stressed and well-watered conditions were considered best performing, and vice versa. The smallest mean difference was observed in SAS183/G/20150, SAS163/P/2015, and SAS137/2015, while the largest mean difference was observed in SAS168/G/2015, SAS160/2015, and SAS183/G/2015. This is summarised in Figure 1.

Effect of repetitive drought on yield

All yield parameters exhibited a highly significant difference among accessions and between water levels

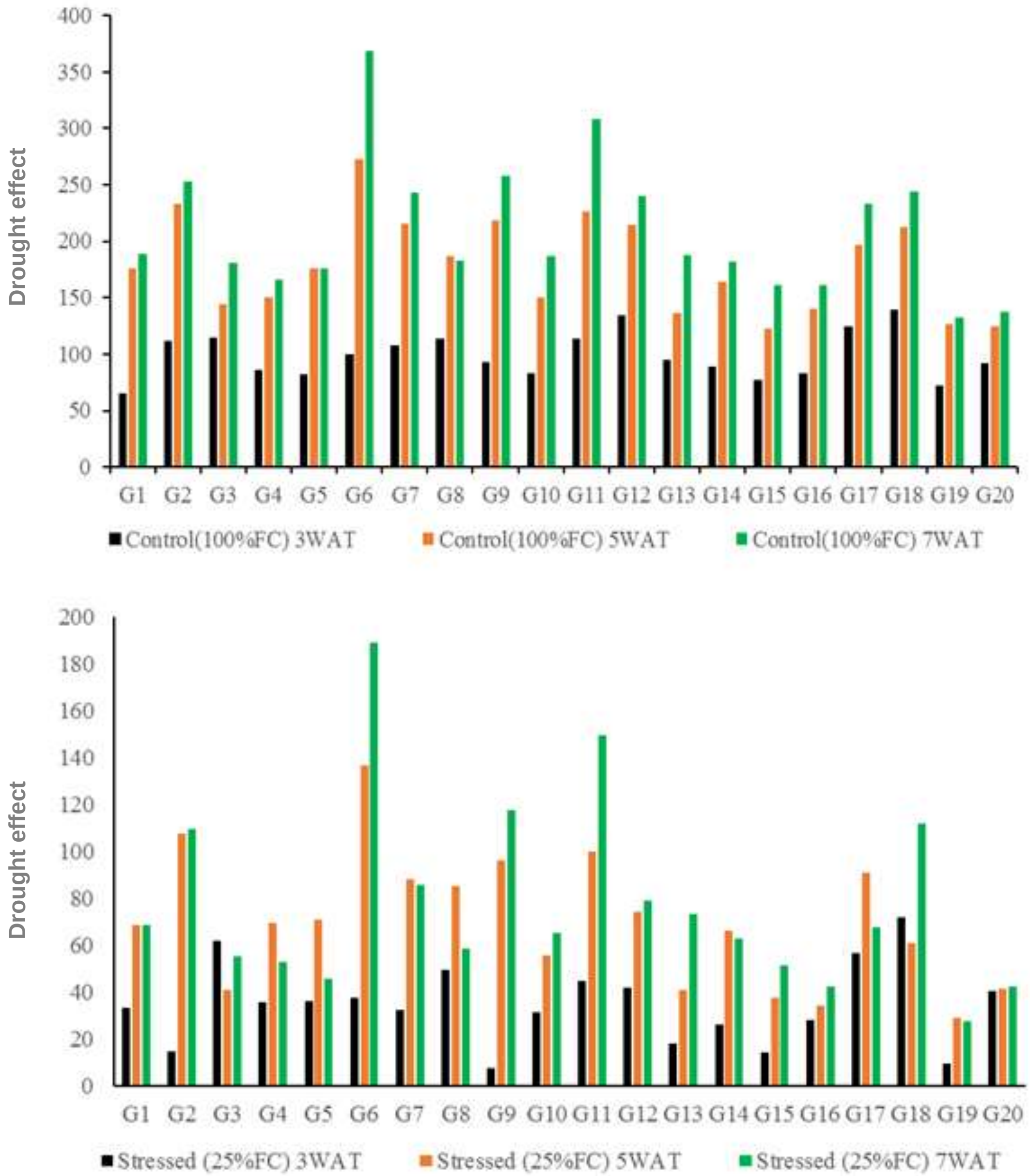

Figure 1. The effect of drought on the growth rate of 20 accessions of *S. aethiopicum* basing on leaf area. G1, SAS168/G/2015; G2, SAS183/G/2015; G3, SAS163/2015; G4, SAS163/P/2015; G5, SAS157/P/2015; G6, SAS160/2015; G7, SAS163/G/2015; G8, SAS183/P/2015; G9, SAS108/2015; G10, SAS157/G/2015; G11, SAS148/2015; G12, SAS145/2015; G13, SAS168/P/2015; G14, SAS184/G/2015; G15, SAS137/2015; G16, SAS184/P/2015; G17, SAS141/2015; G18, SAS108/P/2015; G19, SAS185/G/2015; G20, SAS185/P/2015.

($p<0.001$). The control (100% FC) showed a mean leaf fresh weight of 4.14 g, while the stressed showed a mean of 2.74 g. The mean difference in leaf fresh weight was 1.40 ± 0.53 g, (control minus 25% FC). The control (100% FC) showed a mean fresh shoot biomass of 206.34 g, whereas the stressed showed a mean of 19.93 g. The mean difference in fresh shoot biomass was 186.41 ± 36.43 g (control minus 25% FC). The control (100% FC) showed a mean harvest index of 0.94, whereas the stressed showed a mean of 4.14. The mean difference in harvest index was -3.20 ± 0.95 (control minus 25% FC) with the largest harvest index of 2.09 and the smallest harvest index being -4.66. Therefore, based on the dry shoot biomass the accessions that performed best were SAS137/2015, SAS148/2015, SAS108/P/2015, and SAS160/2015 (Table 3).

Table 3. Mean performance of yield parameters at 7 weeks after transplanting under 25% field capacity.

Accession	Fresh leaf weight			Shoot biomass (fresh)			Harvest index		
	Control	Stressed	Separation of means	Control	Stressed	Separation of means	Control	Stressed	Separation of means
SAS168/G/2015	4.03	2.69	1.34	181.33	18.17	163.17	1.08	4.26	-3.18
SAS183/G/2015	4.83	3.50	1.33	230.67	18.00	212.67	0.84	4.56	-3.72
SAS163/2015	3.05	2.05	1.00	278.17	18.83	259.33	0.50	4.26	-3.76
SAS163/P/2015	4.18	2.75	1.42	158.83	18.17	140.67	0.81	3.89	-3.08
SAS157/P/2015	4.26	2.58	1.68	232.67	20.17	212.50	0.89	4.47	-3.58
SAS160/2015	5.69	3.87	1.82	149.33	19.67	129.67	1.54	5.96	-4.42
SAS163/G/2015	4.73	2.95	1.78	207.40	19.60	187.80	1.01	3.77	-2.76
SAS183/P/2015	3.78	2.44	1.34	254.83	21.17	233.67	0.73	3.42	-2.69
SAS108/2015	3.59	2.52	1.08	206.17	19.17	187.00	0.66	2.75	-2.09
SAS157/G/2015	3.87	2.70	1.17	183.00	20.83	162.17	1.03	4.33	-3.30
SAS148/2015	4.86	3.05	1.81	197.67	16.00	181.67	0.70	2.87	-2.17
SAS145/2015	7.03	3.43	3.60	204.17	23.83	180.33	1.23	3.78	-2.55
SAS168/P/2015	3.09	2.17	0.93	213.67	22.17	191.50	0.70	3.29	-2.60
SAS184/G/2015	4.11	2.74	1.37	259.17	22.00	237.17	0.84	4.44	-3.60
SAS137/2015	3.46	2.41	1.05	156.33	22.00	134.33	1.58	3.88	-2.30
SAS184/P/2015	3.09	2.44	0.65	227.33	20.50	206.83	0.80	3.89	-3.09
SAS141/2015	3.76	2.62	1.14	194.50	20.50	174.00	0.95	3.26	-2.31
SAS108/P/2015	5.51	3.87	1.64	157.50	19.83	137.67	1.00	4.54	-3.54
SAS185/G/2015	3.12	2.20	0.92	218.00	18.83	199.17	0.89	5.55	-4.66
SAS185/P/2015	2.66	1.77	0.88	216.00	19.17	196.83	1.01	5.54	-4.53
F. pr	-	-	<.001	-	-	<.001	-	-	<.001
CV%	-	-	15.1	-	-	12.5	-	-	29.3
S.E	-	-	0.5176	-	-	14.107	-	-	0.744
LSD (5%)	-	-	0.4167	-	-	3.607	-	-	0.1901

DISCUSSION

Effect of repetitive drought on the growth parameters

Drought stress often affects plant morphological traits (Tuberosa, 2012). The studied growth response parameters were number of leaves, plant height, plant canopy, leaf area and plant branching among accessions and the different water levels in the above mentioned traits. Repetitive drought had most effect on growth rate traits at 25 % FC and less effect was observed at 75 % FC. This implies that growth rate is dependent on the level of water stress. Drought has been widely reported to hinder growth (Ekren et al., 2012; Hassan et al., 2012; Muscolo et al., 2015). The growth rate reduction in particular parameters such as leaf area could be a means by which a plant adapts to repetitive drought stress as the plant reduces water loss through evapotranspiration (Ali and Hassan, 2014). The reduced leaf area seems to be the best adaptive trait in response to repetitive drought stress in *S.*

aethiopicum Shum group accessions. The reduction in the number of leaves under repetitive drought stress is another measure plants use to reduce the surface area available for transpiration as reported in willows (Pucholt et al., 2015). There was an effect of repetitive drought on plant height at 25% FC. This is possibly due to decrease in both cell growth and development as a result of decrease in turgor (Ali and Hassan, 2014). According to Ali and Hassan (2014), the hindrance in cell growth leads to a decrease in plant height. There was no effect of repetitive drought on plant branching. However, there was a significant difference among accessions, without regard to moisture stress level. This finding is similar to the observations made in *Coriandrum sativum* by Ali and Hassan (2014). This suggests that plant branching as a trait may be more influenced genetically than by the environment.

There was significant growth for all accessions as measured using different traits from one to another phase of re-watering. This shows that a small rainfall pulse could induce a rapid response which quickly triggers plant growth in order for plants to survive. This implies that *S. aethiopicum* Shum group could be a suitable crop for drought-prone agro-ecologies (Mbadianya et al., 2013). Plants at 7 WAT were more affected by the repetitive drought than the plants at 3 WAT. Therefore, the morphological adjustment of the plant depends on the growth stage (Altmann et al., 2015).

A highly significant variation among accessions ($p<0.001$) implies that the study traits can be used in the discrimination of accessions as suggested earlier by Adeniji et al. (2013) and Sseremba et al. (2017). Different accessions performed differently for the different parameters. This portrays a picture that different accessions could be using different mechanisms of drought tolerance such as escape, avoidance, tolerance, and recovery (Kumar et al., 2012).

The principal component analysis showed that leaf area at 7 WAT contributed the highest to variation among the study accessions. This may imply that leaf area at 7 WAT may be a good indicator to use in the selection for repetitive drought tolerance in *S. aethiopicum* Shum group.

Effect of repetitive drought on yield

The differential performance of accessions for different yield-related traits suggests a need to identify suitable yield parameters for standardizing selection criteria under drought stress. The observed accession differences could be attributed to genotype-environment interaction as different accessions may have different adaptive mechanisms to drought (Abakemal et al., 2016). Drought stress decreased all the yield parameters except harvest index which is contrary with what was reported in maize cultivars (Khalili et al., 2013).

Production of leafy vegetables is severely affected by soil water status (Kumar et al., 2012). A moderate level of soil water availability usually ensures an optimal yield. The reduction in leaf weight, as observed from this study, is an indicator that plants responded to drought stress by reducing the available surface area for transpiration (Chatterjee and Solankey, 2015). It could also be as a result of a reduction in the chlorophyll content as reported by Hailemichael et al. (2016). The reduction in the shoot biomass is an indication that shoots are greatly affected by drought stress. This occurs when the plants tend to concentrate on root development for water and available nutrients, while reducing canopy cover as a survival mechanism; consequently, resulting in an increased root/shoot ratio (Basu et al., 2016).

Unexpectedly, the harvest index in the stressed condition was higher than that in the control treatment. This was because of the high fresh shoot biomass, mainly contributed by the non-harvestable stem mass for the plants under well-watered condition (Kumar et al., 2012; Basu et al., 2016).

Conclusion

The study showed that repetitive drought stress has significant effects on both growth rate and yield. Therefore, the hypothesis that states there is no significant difference among Shum group accessions in their responses to repetitive drought stress is rejected. This study also provided substantial information concerning the variability of the study accessions. It was noted that considerable variation in tolerance to repetitive drought tolerance exists in the evaluated *S. aethiopicum* Shum germplasm. Based on growth parameters, accessions SAS108/2015, SAS163/P/2015, SAS183/G/2015, and SAS168/G/2015 were the most tolerant to repetitive drought stress. Additionally, accessions SAS137/2015, SAS148/2015, SAS108/P/2015 and SAS160/2015 were the most tolerant to repetitive drought stress, based on yield-related traits.

Whereas the focus of this study was on morphological traits only, drought stress effects are first manifested at physiological, biochemical and genetic levels. It is therefore important that complementary studies focusing on the physiological, more morphological and molecular responses to repetitive drought stress are carried out. However, the observed variation in yield and different growth parameters among the accessions shows promise for improvement of drought tolerance in *S. aethiopicum* Shum.

Further, since drought recovery, drought tolerance and yield are quantitative traits, they are subject to genotype by environmental interaction (Kumar et al., 2012), and follow-up from field multi-location evaluations are necessary.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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