Evaluation of image-based phenotyping methods for measuring water yam (*Dioscorea alata* L.) growth and nitrogen nutritional status under greenhouse and field conditions

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Supplementary materials
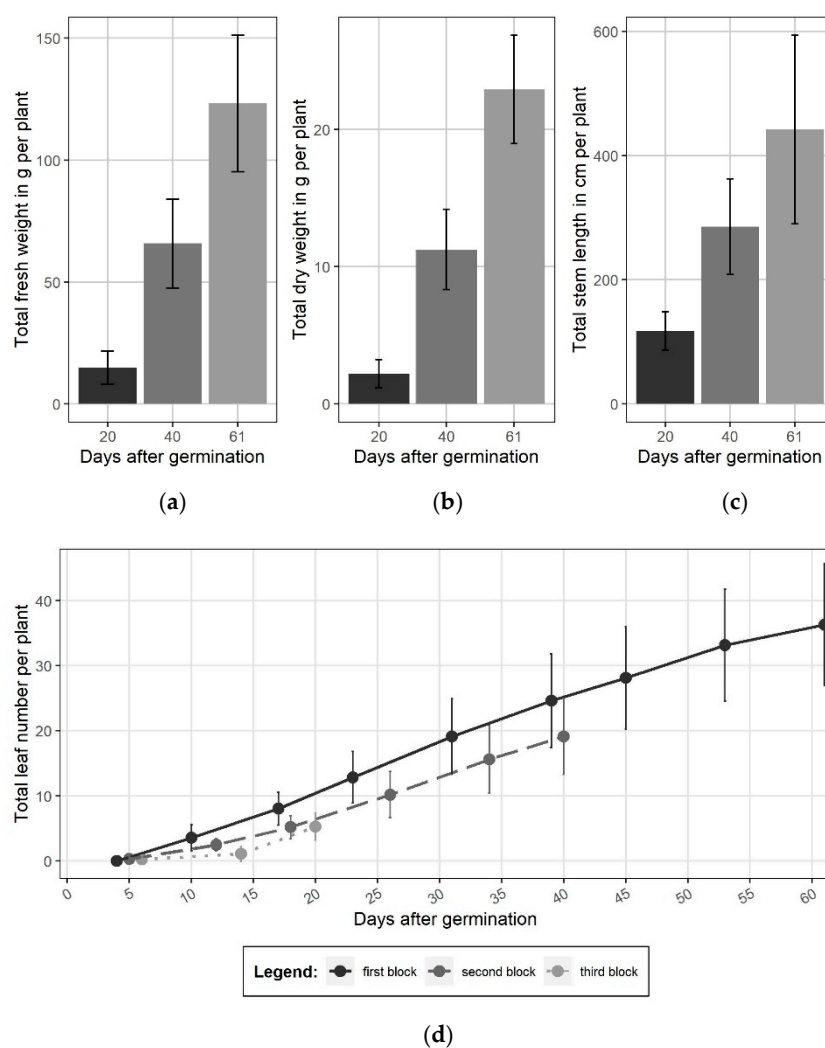
1. Supplementary materials on plant growth and N leaf content of the 1st greenhouse experiment [24]

We observed continuous increases in water yam aboveground fresh and dry matter, stem length, and number of leaves until 61 days after emergence (Figure S1).


(a)   (b)   (c)

(d)

Figure S1. Changes with time in (a) total aboveground fresh weight in g per plant, (b) total aboveground dry weight in g per plant, (c) total stem length in cm per plant, and (d) total number of leaves per plant in water yam (cv raja ala) grown for 61 days after emergence in a greenhouse in the presence of different N inputs. Each column represents the average measured for 20 plants, the bars show the standard deviation.

Nitrogen fertilization had no effect on the total fresh weight at 20 days after emergence but had a significant effect at 40 and, 61 days after emergence (Table S1). Nitrogen concentration of the 1st and 7th fully developed leaves sampled from the apex of the main vine decreased from 20 to 61 days after emergence and N concentration was lower in the 7th leaf compared to the 1st leaf. Whereas N fertilization had little impact on the leaf N concentration at 20 days after emergence, increased N inputs led to increased N leaf content after 40 and, 61 days after emergence (Figure S2).

Table S1. Effect of N fertilization on total aboveground fresh weight (in g per plant) of water yam (cv raja ala) grown for 20, 40 and 61 days after emergence in a greenhouse. Different letters within a column indicate significant differences according to a Fisher's least significance test at *p*-level ≤ 0.05 (n = five plants per treatment).

N fertilization [kg N ha^{-1}]	Total aboveground fresh weight g plant^{-1}		
	20 days after emergence	40 days after emergence	61 days after emergence
0	14.16 a	48.38 b	92.66 b
50	10.02 a	66.72 ab	132.66 a
100	17.36 a	78.70 a	126.70 a
170	15.60 a	69.52 a	140.82 a
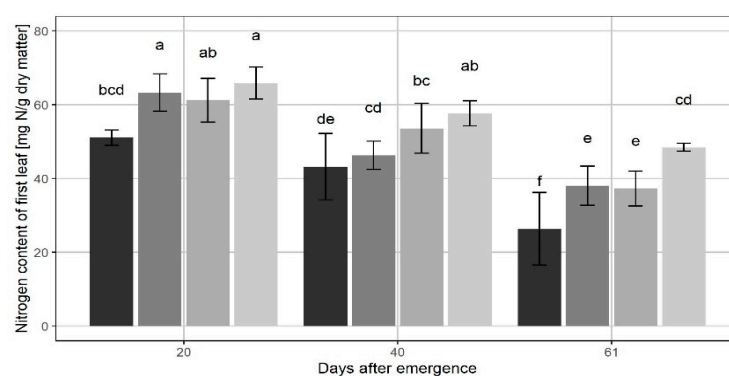
(**a**)
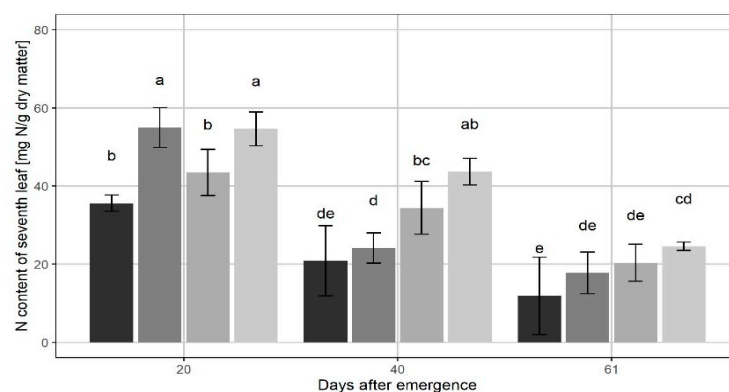
(**b**)

Figure S2. Nitrogen concentrations in the 1st (**a**) and 7th (**b**) fully developed leaves sampled from the apex of the main vine after three, six and nine weeks after germination (20, 40 and 61 days after emergence) in water yam (cv raja ala) grown in a greenhouse in the presence of different N fertilization rates. Columns show the average and the bar the standard deviation values. Letters denote significant differences calculated with least significant difference (LSD) test at *p*-level ≤ 0.05 (n = five plants per column).

2. Supplementary materials on the 1st greenhouse experiment [22]

This experiment was conducted at the ETH research station for plant sciences in Lindau-Eschikon, Switzerland, from July to October 2015.

2.1. Materials and Methods

2.1.1. Plant materials, experimental design and plant growth conditions

Two genotypes of water yam were selected for the pot experiment. One genotype (SL, cv. Raja ala), a purple-fleshed variety with purplish veins, stems and young leaves, was obtained from Sri Lanka, but sourced locally at SK Trading GmbH, Zurich, Switzerland. The second genotype (CI, cv. Florido), a white fleshed variety with purely green leaves, was obtained from a local market in Youpougon, Abidjan, Côte d'Ivoire. Both genotypes were received in June 2015.
Tubers of both genotypes were cut into setts of 100 g fresh weight on July 7th 2015. The round tubers of SL were halved into head (proximal) and tail (distal) parts and each half was then cut into wedge-shaped pieces. The oblong tubers of IC, were first halved lengthways, and then cut into several head, middle and tail pieces. Cut setts were immersed in a liquid mixture of a broad-spectrum fungicide (active ingredient: 70% mancozeb, 1.75% bentihavalicarb) and insecticide (active ingredient: 25% diazinon) and were air-dried for 20 hours. A total of 24 setts per genotype was planted in the pots and substrate described in the main part of the paper. At planting, setts were assigned to a nitrogen (N) treatment. Head, middle and tail setts, as well as setts originating from different tubers (with potential differences in dormancy) were equally distributed among N treatments. Pots were then placed in a greenhouse chamber in a randomized complete block design, consisting of four blocks (i.e. four replicates à 12 pots/block: two genotypes, three N treatments, two plant sampling dates 6 and 8 weeks after emergence). The environmental conditions were the same as those described in the main text of the paper.
Plant emergence was recorded for each individual plant, when the first sprout became visible. All 48 plants emerged between two and six weeks after planting. Original sett position within the tuber (head, middle, tail) strongly affected the emergence date, with head setts emerging faster (2 to 3 weeks after planting) than middle and tail setts (3 to 6 and 4 to 6 weeks after planting, respectively). Plants were grouped into emergence classes, as suggested by Cornet et al. (2014) to reduce the high plant variability characteristic for yam. Plants emerging within the same week were grouped into the same class. Timing of fertilizer applications and measurements was defined with regard to emergence classes
Three N levels were considered for each genotype, either no N added (0N) or an application of 101 or 342 mg N pot^{-1} (corresponding to 50 (50N) and 170 kg N ha^{-1} (170N), respectively). N was added two weeks after emergence (wae) as $Ca(NO_3)_2 \cdot 4H_2O$ for both treatments. Nitrogen was applied once in the 50N treatment and was split at two and four wae for 170N. Further nutrients were added in a nutrient solution 2 wae as follows: 175 mg P as KH_2PO_4; 35 mg S as K_2SO_4; 3.5 mg Zn as $ZnSO_4 \cdot 7H_2O$; and 3.5 mg Fe as Fe-EDTA per pot. The total amount of K applied as KH_2PO_4 and K_2SO_4 was 263.6 mg K pot^{-1}.

2.1.2. Measurements

2.1.2.1. Measurements on plant level

Total shoot length (sum of both main and lateral shoots) was measured using a flexible tape. Shoot length of main shoots was measured from the substrate surface to the tip of the apical bud, that of lateral shoots from the axil (on the main shoot) to the tip of the apical bud.
The total number of unfolded leaves per plant was counted. Unfolded leaves were defined as those with a smooth and flat lamina. Counting leaves proved difficult for many plants of CI as they showed symptoms indicating a virus infection. Symptoms included stunted growth and a range of symptoms on the leaves, including: mosaic pattern, deformation (crinkling and blistering) and early senescence.

As plants were mainly affected by virus infection within their first few weeks of growth, symptoms were only found on older leaves at harvest. Deformed leaves never met the actual definition of an unfolded leave, but they were nevertheless counted as one since they were clearly unfolded with regard to their development stage.

Total leaf area (LA) was defined as the leaf area of all unfolded leaves. Leaf blades of unfolded leaves were cut and total leaf area was measured by scanning them with a leaf area meter (LICOR 3100, LI-COR, Lincoln, NE, USA). Shoot fresh weight (FW) was determined immediately after cutting the stems at soil surface. Shoot dry weight (DW) was obtained after drying the shoots to constant weight at 60°C for three days. Both fresh and dry weight were determined with a precision scale (PM4600 DeltaRange, Mettler-Toldo GmbH, Greifensee, Switzerland, 0.01g precision).

Image-based phenotyping at plant level was conducted as described in the paper.

2.1.2.2.　Measurements on leaf level

Measurements on leaf level were only performed on one vine per plant. For plants with several vines, only the longest was selected for measurements. All measurements were taken on every 8th unfolded leaf of the main shoot– counting nodes from the base of the vine to the apex (i.e. leaves at the 1st, 8th and for some plants 16th node). Most plants had an alternate leaf arrangement on the lower part of the stem, but an opposite leaf arrangement on the upper part. In an opposite pattern, selection of the measured leaf was random.

SPAD data and N leaf content were acquired on these leaves as described in the paper.

Image-based phenotyping at leaf level was conducted as described in the paper.

2.2. *Main results*

Table S2. Pearson's correlation coefficient of projected leaf area assessed from the nadir (top) view, side view 0°, side view 90°, the sum of two views (nadir and side 0°) and the sum of three views and destructively measured total leaf area, dry weight and fresh weight measured on two genotypes of water yam (raja ala and florido, n=24 genotype^{-1}) grown in the greenhouse under three nitrogen fertilizer treatments (0, 50 and 170 kg N ha^{-1}) and harvested six and eight weeks after emergence. Leaf area was transformed prior to the calculation using the natural logarithm.

	Leaf area [cm²]	Dry weight [g]	Fresh weight [g]
Projected leaf area [cm²]			
top-view	0.87***	0.76***	0.80***
side-view 0°	0.86***	0.83***	0.82 ***
side-view 90°	0.88***	0.87***	0.84***
sum of 2	0.93***	0.86***	0.87***
sum of 3	0.94***	0.89***	0.89***

*Significance code: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1*
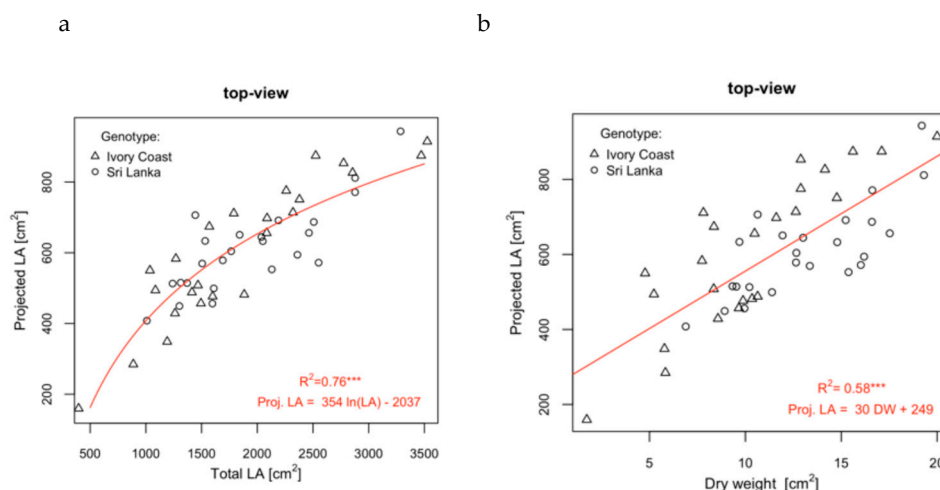
Figure S3. Relation between projected leaf surface calculated from single top (nadir) images, and destructively measured total leaf surface a), and shoot dry weight b) for two genotypes of water yam (SL, CI, n=24 genotype^{-1}) grown in the greenhouse under three nitrogen fertilizer treatments (0, 50 and 170 kg N ha^{-1}) and harvested six and eight weeks after emergence.
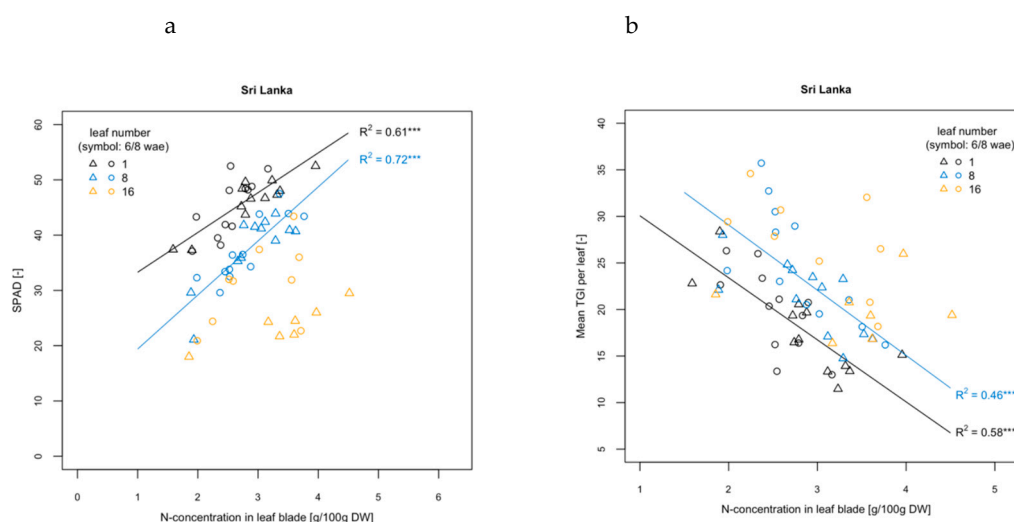
Figure S4. Relation between SPAD value and leaf nitrogen (N) content at different leaf positions (leaf no. 1, 8, 16, counted from base to apex) a) and between TGI value (triangular greenness index) and leaf nitrogen (N) content at different leaf positions (leaf no. 1, 8, 16, counted from base to apex) b) in water yam cv raja ala, six and eight weeks after emergence (wae). Plants were grown under three nitrogen treatments (0, 50, 170 kg N ha^{-1}) in a greenhouse. Regression lines were included, when results were significant (p-value <= 0.05).

3. Supplementary materials for the field experiment in Tieningboué
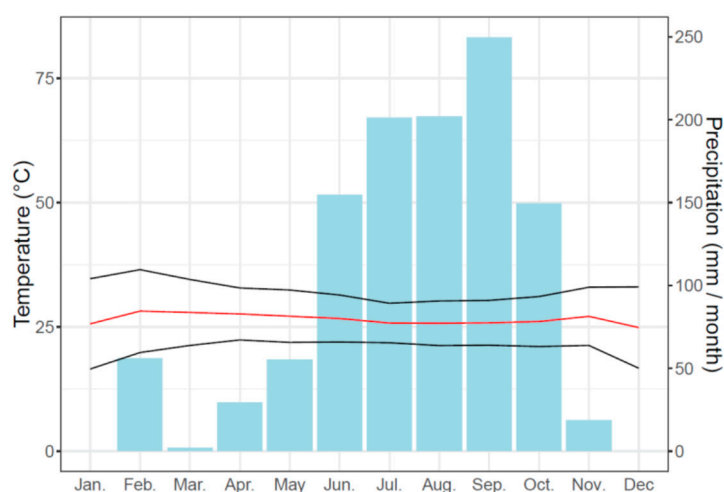
Figure S5. Climate diagram of the season 2018 for Tieningboué. The mean temperature (°C) is shown by the red line, the maximum temperature by the upper black line and the minimum temperature by the lower black line. The precipitation (mm) is shown with bar plots. No weather data was available for 22 days in March and 22 days in April 2018

Table S3. Tuber yield (average and standard error) of water yam cv C18 measured in a nearby experiment located on the same soil and submitted to the same treatments (rotation R1 to R4 and fertilization T0 to T3, see details in the paper) as those studied in our field experiment.

| Rotation	Fertilization	yield t fresh tuber ha^{-1}	
		average	stdev
R1	T0	10.85	1.67
R1	T1	7.44	1.56
R1	T2	13.34	2.51
R1	T3	12.15	2.09
R2	T0	8.15	0.95
R2	T1	10.94	1.34
R2	T2	11.06	1.48
R2	T3	11.88	2.2
R3	T0	9.04	2.28
R3	T1	9.44	0.71
R3	T2	7.67	1.57
R3	T3	9.94	3.47
R4	T0	6.97	1.7
R4	T1	12.48	1.06
R4	T2	7.67	1.57
R4	T3	9.94	3.47

Table S4. Soil surface coverage (%) measured from pixel numbers obtained from TGI image for each studied plant between July 5, 2018 and August 10, 2018 in our field experiment.

Plot ID	Plant ID	Rotation	Fertilization	Days after emergence						
				7	13	16	19	23	41	43
R1T0_2	2	R1	T0	3.91	3.17	3.46	5.07	5.50	13.16	13.53
R1T0_4	4	R1	T0	2.98	3.48	4.88	7.39	10.40	20.54	19.32
R1T1_3	3	R1	T1	6.64	9.35	12.43	13.92	18.42	26.62	27.39
R1T1_4	4	R1	T1	1.57	6.16	7.77	9.20	10.60	22.71	19.67
R1T2_3	3	R1	T2	2.26	4.85	7.22	10.32	12.15	29.71	30.89
R1T2_6	6	R1	T2	3.38	3.69	7.06	8.64	15.06	25.80	28.82
R1T3_2	2	R1	T3	2.18	3.98	8.27	8.44	9.45	16.76	14.76
R1T3_5	5	R1	T3	1.15	2.79	3.43	5.36	5.45	15.25	13.53
R2T0_2	2	R2	T0	3.58	2.45	3.78	5.22	7.47	11.75	11.60
R2T0_6	6	R2	T0	3.44	4.52	5.49	7.57	9.39	16.89	15.94
R2T1_1	1	R2	T1	4.19	4.50	7.29	9.16	12.00	20.22	19.46
R2T1_2	2	R2	T1	2.62	3.29	4.88	6.27	9.08	16.45	15.19
R2T2_1	1	R2	T2	4.61	5.00	6.58	9.15	16.95	20.42	21.31
R2T2_4	4	R2	T2	5.85	7.78	13.51	21.38	23.26	30.13	28.47
R2T3_3	3	R2	T3	4.07	5.97	9.70	10.23	15.92	27.27	27.49
R2T3_4	4	R2	T3	2.82	5.44	7.82	8.78	11.91	22.80	21.61
R3T0_1	1	R3	T0	2.43	3.52	3.39	3.92	6.29	12.60	10.90
R3T0_6	6	R3	T0	4.91	6.91	5.67	6.50	11.99	14.82	17.08
R3T1_2	2	R3	T1	2.53	3.93	5.49	5.90	7.98	14.60	12.71
R3T1_6	6	R3	T1	2.22	3.33	4.01	6.72	7.34	17.16	16.48
R3T2_2	2	R3	T2	2.82	5.32	5.25	7.64	10.12	14.55	15.62
R3T2_4	4	R3	T2	4.01	6.68	7.05	10.89	13.26	18.66	15.42
R3T3_1	1	R3	T3	0.60	4.28	6.99	10.08	13.12	22.34	21.64
R3T3_6	6	R3	T3	1.04	1.42	2.26	3.06	4.92	13.89	12.83
R4T0_4	4	R4	T0	3.51	1.97	7.56	11.49	13.20	25.83	19.39
R4T0_5	5	R4	T0	3.00	4.51	3.09	4.76	4.95	9.23	9.43
R4T1_1	1	R4	T1	4.84	5.30	7.75	9.63	10.44	18.42	18.23
R4T1_2	2	R4	T1	2.07	4.99	7.21	8.09	10.00	24.12	23.04
R4T2_2	2	R4	T2	1.70	1.28	3.68	6.17	7.71	13.58	12.20
R4T2_4	4	R4	T2	2.44	5.20	7.18	8.46	15.40	25.67	24.39
R4T3_1	1	R4	T3	0.80	1.83	4.62	7.71	10.36	24.81	22.63
R4T3_5	5	R4	T3	2.66	3.26	6.32	7.51	11.20	21.50	25.03
			average	**3.03**	**4.38**	**6.28**	**8.27**	**10.98**	**19.63**	**18.94**
			stdev	**1.41**	**1.82**	**2.53**	**3.34**	**4.15**	**5.59**	**5.84**

Table S5. Average and standard deviation values of the slope (a, expressed in SSC day^{-1}) of the linear equations describing the changes in soil surface cover by yam shoots with time between July 5 and August 10 for the various fertilization and rotation treatments implemented in a field experiment conducted with water yam (cv C18) in Tieningboué (Côte d'Ivoire). Each treatment has a n = 8 plants. Due to the absence of replicate, it was not possible to analyze these data statistically.

Treatments	a	
	average	stdev
Fertilization		
T0	0.361	0.133
T1	0.548	0.206
T2	0.556	0.196
T3	0.540	0.139
Rotation		
R1	0.538	0.184
R2	0.567	0.221
R3	0.384	0.097
R4	0.517	0.183
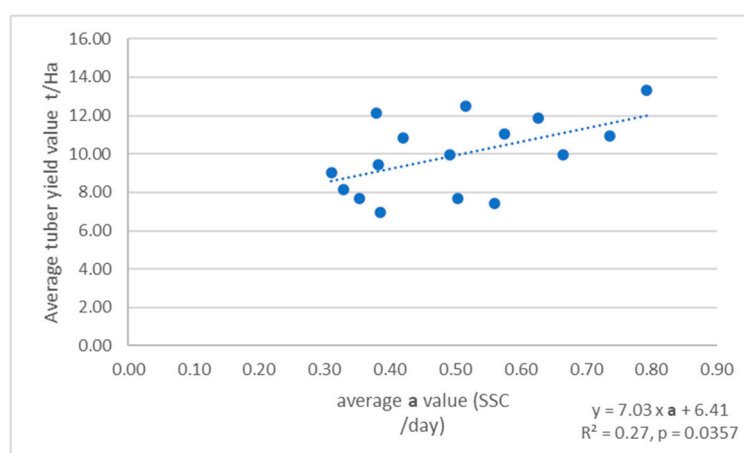
Figure S6. Relationship between final tuber yields and the average daily rate of soil surface coverage by yam shoots (**a**) between July 5, 2018 and August 10, 2018 in our field experiment.

Table S6. TGI measured at plant level for each studied plant between July 5, 2018 and August 10, 2018 in our field experiment.

Plot ID	Plant ID	Rotation	Fertilization	Days after mergence						
				7	13	16	19	23	41	43
R1T0_2	2	R1	T0	4.71	4.17	3.13	3.21	2.70	3.09	3.04
R1T0_4	4	R1	T0	4.55	3.70	3.06	3.24	3.00	2.75	2.69
R1T1_3	3	R1	T1	4.89	4.07	3.81	3.36	3.33	2.97	2.94
R1T1_4	4	R1	T1	4.20	5.34	4.17	4.01	3.68	3.23	3.34
R1T2_3	3	R1	T2	4.92	3.87	3.79	3.88	3.41	2.76	2.95
R1T2_6	6	R1	T2	4.26	4.96	2.85	2.98	3.04	2.64	2.71
R1T3_2	2	R1	T3	4.48	4.32	4.43	3.87	3.27	3.03	3.01
R1T3_5	5	R1	T3	4.46	4.39	3.64	3.64	2.81	2.96	2.69
R2T0_2	2	R2	T0	4.37	4.69	3.74	3.92	3.31	3.51	3.03
R2T0_6	6	R2	T0	4.62	3.87	3.20	3.26	3.21	3.38	2.88
R2T1_1	1	R2	T1	4.74	4.71	3.75	3.16	3.01	2.72	3.08
R2T1_2	2	R2	T1	4.68	4.65	3.45	3.11	3.22	3.01	3.28
R2T2_1	1	R2	T2	4.90	3.60	2.94	3.47	3.24	3.12	2.83
R2T2_4	4	R2	T2	4.51	3.75	3.40	3.83	3.24	3.20	2.54
R2T3_3	3	R2	T3	4.74	4.01	3.44	3.12	2.83	2.65	2.80
R2T3_4	4	R2	T3	4.85	4.85	3.81	3.37	2.99	2.93	2.86
R3T0_1	1	R3	T0	4.23	4.64	3.63	3.02	3.15	3.11	3.04
R3T0_6	6	R3	T0	4.67	4.25	3.30	3.15	3.05	3.22	2.58
R3T1_2	2	R3	T1	4.58	4.62	3.71	3.60	2.85	2.84	2.77
R3T1_6	6	R3	T1	3.93	3.98	3.50	3.68	2.84	3.22	2.83
R3T2_2	2	R3	T2	3.48	4.33	3.19	3.29	3.23	2.75	2.69
R3T2_4	4	R3	T2	4.27	4.62	3.20	3.68	3.29	2.85	2.60
R3T3_1	1	R3	T3	4.53	5.21	4.21	4.78	3.38	3.21	3.19
R3T3_6	6	R3	T3	4.57	6.13	4.16	5.17	3.83	3.04	2.86
R4T0_4	4	R4	T0	4.58	3.51	3.93	4.03	3.24	3.19	3.01
R4T0_5	5	R4	T0	4.89	3.44	3.48	4.11	3.11	3.22	2.76
R4T1_1	1	R4	T1	4.73	3.66	4.00	4.03	3.20	3.17	2.87
R4T1_2	2	R4	T1	4.82	4.83	4.11	3.74	3.28	3.08	2.76
R4T2_2	2	R4	T2	5.25	3.49	4.00	4.33	3.09	3.09	2.69
R4T2_4	4	R4	T2	4.85	3.80	3.20	3.83	2.81	3.05	2.75
R4T3_1	1	R4	T3	4.08	3.84	3.48	5.22	3.82	3.70	3.15
R4T3_5	5	R4	T3	4.21	3.22	3.38	4.58	3.37	3.56	3.05
			average	**4.55**	**4.27**	**3.60**	**3.74**	**3.18**	**3.07**	**2.88**
			stdev	**0.34**	**0.64**	**0.40**	**0.59**	**0.27**	**0.25**	**0.20**