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EVALUATION OF LEAF-WATER RELATION TRAITS, AS SELECTION CRITERION FOR DEVELOPING DROUGHT RESISTANT POTATO (*Solanum Tuberosum L.*) GENOTYPES

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ABSTRACT

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Though breeding for drought resistance is tricky due to the many physiological and biochemical processes involved and their interaction with the environment; availability of precise, cheap and easy to apply selection tool is critical. The present study quantified the response of potato genotypes to drought and identified potential leaf-water relation traits that enables for identifying drought resistant genotypes. The study assessed sixty genotypes under two irrigation regimes: fully watered non-stress and terminal drought, whereby irrigation was withheld after 50 % flowering to induce post-flowering stress. Measurements for various traits were taken following the potato crop trait ontology. The post-flowering stress induced in this study caused a tuber yield reduction of 33.13% compared with the non-stressed treatment. The genotypes responded differently in tuber yielding potential to the drought. This differential tuber yield response to drought was associated with up and downward regulation of multiple traits related to drought adaptation in potatoes. Drought caused downward regulation on trait responses such as harvest index, leaf area, and specific leaf area. Aboveground biomass and relative water content of leaf contributed negatively for tuber yield under stressed condition. Hence, the attributes identified from this study could help the potato breeding program on drought resistance to develop climate resilient potato varieties.

Contribution/Originality: This study contributes in the existing literature vis-à-vis drought adaptive leaf-water relation traits and is one of the very few studies which have evaluated potato genotypes for terminal drought stress. The study assessed sixty genotypes by scientifically comparing them with very important traits and come up with valid conclusion.

1. INTRODUCTION

The increasing awareness on its productive capability and food value placed potato (*Solanum tuberosum L.*) among the most important staple food crops in the world. In recent years, its production has been increased substantially throughout Ethiopia from mid to high altitude areas and in lowlands to some extent both under rain-fed and irrigated conditions. The average productivity of potato at farmers' field is very low i.e 12.3 tha^{-1} [1] which is only about one-fourth of achievable yields (34-47 tha^{-1}) at research stations. Diverse and complex biotic, abiotic, and anthropogenic factors have contributed to the gap between the attainable potential yield and the existing low productivity of potato in Ethiopia. Among the abiotic stresses, drought is the most complex and serious danger to

global agricultural production [2]. Potatoes regularly undergo through a short-lived water shortage in most of the rain-fed growing regions due to erratic rainfall or inadequate supplemental irrigation techniques [3]. Cut-off rain late in the growing season is the major cause for potatoes to suffer from drought in the major production ecologies in Ethiopia [4]. Moreover, drought resistance is amongst the priority attribute growers make choice for cultivars to plant with. This problem can be mitigated by breeding varieties which can produce stable yields in water limiting conditions. However, different crops respond differently to water stress with respect to various morphological, physiological and yield related traits. Hence, identification of easily measurable drought adaptive traits with good degree of heritability and higher degree of association with improved yield under drought is critical for developing successful breeding program. Some leaf-water relation traits of plants have been advocated as indicators for the development of drought resistant genotypes i.e. relative water content of leaf [5] and excised leaf water loss [6]. However, similar works on potato was minimal. Moreover, in the face of climate change and population growth, it needs to develop drought adaptive potato cultivars. Accurately identifying drought resistance traits and understanding the interaction amongst these traits with tuber yield are pertinent inquiries to be addressed. Therefore, this particular study was carried to evaluate the response of potato genotypes for post flowering drought stress and identify leaf-water relation traits en route for identifying drought resistant genotypes.

2. MATERIALS AND METHODS

2.1. Description of the Study Area

The study was conducted under irrigated condition in 2015/16 at Koga trial site of Adet Agricultural Research Center located in Amhara National Regional State, Ethiopia. It has got an elevation of 1960 meter above sea level. Its soil represents a heavy clay-textured red Nitisol. The detailed climatological descriptions of the study area are indicated below in Table 1.

Table-1. Metrological description of the study area during the cropping months.

Parameters	January	February	March	April	Mean
Minimum temperature (°C)	7.40	8.73	10.44	12.17	9.69
Maximum temperature (°C)	28.60	31.32	30.89	30.56	30.34
Mean rain fall (mm)	3.43	4.88	15.13	44.51	16.99
Relative humidity (%)	48.19	44.25	42.06	42.35	44.21
Sunshine hours (hr.)	9.51	8.99	8.78	8.73	9.00

Source: Soil and water research directorate, Adet Agricultural Research Center.

2.2. Experimental Materials

The experiment was conducted using a total of 60 potato clones of which 52 are introduced from CIP (*Centro Internacional de la Papa*), Lima, Peru, six are released varieties in Ethiopia, one introduced variety and one is a local check cultivar Table 2.

Table-2. List of potato genotypes used in the study.

No	Genotype ID	No	Genotype ID	No	Genotype ID	No	Genotype ID
1	Guassa	16	CIP-301044.36	31	CIP-398208.29	46	CIP-396036.201
2	CIP-304345.47	17	CIP-398180.612	32	CIP-398190.53	47	CIP-399048.24
3	CIP-396038.101	18	CIP-397069.5	33	CIP-397029.21	48	CIP-399001.44
4	Belete	19	CIP-396046.105	34	CIP-391011.17	49	Gudene
5	CIP-393077.54	20	CIP-394898.13	35	CIP-396272.21	50	CIP-397014.2
6	CIP-391045.74	21	CIP-381379.12	36	CIP-384866.5	51	CIP-300054.29
7	CIP-304356.32	22	CIP-310135.14	37	CIP-398208.704	52	CIP-395015.6
8	CIP-395169.17	23	CIP-395077.12	38	Gorebella	53	CIP-391580.3
9	CIP-398089.119	24	CIP-301024.14	39	CIP-396027.205	54	CIP-399085.17
10	CIP-396285.1	25	CIP-301040.63	40	CIP-391065.69	55	CIP-300099.22
11	CIP-391533.1	26	CIP-301024.95	41	CIP-396004.225	56	CIP-304371.67
12	CIP-392639.34	27	Jallene	42	CIP-398190.735	57	CIP-302499.3
13	CIP-398190.605	28	CIP-302498.7	43	CIP-398193.65	58	CIP-396272.37
14	CIP-380011.12	29	CIP-396037.215	44	Granola	59	Shenkolla
15	CIP-393227.66	30	CIP-398192.41	45	CIP-397054.3	60	Ater abeba

2.3. Experimental Design and Management

The experiment was laid out in a 10 x 6 alpha lattice design with two replications and under two moisture regimes (stressed and non-stressed). Well-sprouted seed tubers were planted at a spacing of 75cm between rows and 30 cm between plants on a plot. All other standard agronomic operations such as earthing-up, weeding, and fertilization were uniformly carried-out over entire experimental plot irrespective of water regime.

Under non-stressed treatment, genotypes were regularly watered using surface furrow irrigation at a week interval until physiological maturity, while in the stressed treatment; the genotypes were regularly irrigated at a week interval till 50 % of the genotypes initiated flowering and then totally cut-off irrigation water supply till the end of maturity starting from 50% flowering stage of each treatment. The non-stress trial received 6-8 times irrigations between flowering and physiological maturity to ensure optimum crop growth. The stress plots were covered with a movable rain out shelter when the rain seems to shower.

2.4. Soil Moisture

Soil moisture during the plant growth period was recorded by installing a Watermark Meter (*Model 2000ss, IRRROMETER Company, INC, USA*) on 12 representative points across the stress and non-stress fields. Measurements were taken at a soil depth of 15, 30 and 45cm during various growth stages *i.e.* at full emergence, 50 % flowering, 15 days from the onset of the stress, 30 days from onset of the stress and at physiological maturity.

2.5. Evaluation of Leaf-Water Relation and Yield Related Traits

Data on leaf-water relation and yield related traits such as excised leaf water loss, relative water content of leaf, harvest index, and above ground biomass, leaf area and specific leaf area was measured following the standard data collection protocols described below:

Above ground biomass per plant: above ground parts (stem, branch, and leaves) of three randomly selected plants were harvested from each treatment category when the vines still green but had practically ceased growth (50 % of the leaves turned yellowish). Dry mass was determined after drying the samples in an oven at 72 °C to a constant weight and averaged to find above ground biomass per plant basis. Leaf area: three leaves per plant were collected from three comparative plants in a plot and leaf area was estimated using a portable leaf area meter (*AM300 Area Meter, ADC BioScientific Ltd.*) and expressed as mm² at about 20 days after inducing the stress. Specific leaf area: leaf samples collected for leaf area measurement were oven dried till a constant weight was achieved. Then the leaf dry matter was obtained by weighing the samples on a sensitive balance. Specific leaf area was calculated as the ratio of leaf area to leaf dry weight.

Excised leaf water loss: calculated as:

$$\text{Excised leaf water loss} = \frac{\text{fresh leaf weight} - \text{two hours dry leaf weight}}{\text{dry leaf weight}}$$

The procedures were as follows, six fully expanded leaves were randomly sampled from each plots after 20 days of imposing the stress. The fresh weight was measured immediately and the leaves left to wilt for two hours under room temperature and then weighed. The leaves were then oven dried at 80 °C for twenty four hours and the dried weight was measured. Relative water content of leaf: calculated as;

$$\text{Relative water content of leaf} = \frac{\text{fresh leaf weight} - \text{dry leaf weight}}{\text{turgid leaf weight} - \text{dry leaf weight}} \times 100$$

Where by, DW was determined by oven drying of leaves at 80 °C for 24 h, and TW was determined from plants that have been watered and kept overnight in petri- dishes.

Harvest index: calculated as

$$\text{Harvest Index} = \frac{\text{Economic yield}}{\text{total biomass}} \times 100$$

2.6. Data Analysis

a. Analysis of Variance

The collected data was analyzed using the linear model in alpha lattice design using SAS 9.1 statistical software and mean comparisons were done using Duncan Multiple Range Test.

$$Y_{ijk} = \mu + t_i + r_j + B_{k(j)} + E_{ijk}$$

Where, Y_{ijk} denotes the value of the observed trait for i^{th} treatment received in the k^{th} block within j^{th} replicate (superblock), t_i denotes the fixed effect of the i^{th} treatment ($i = 1, 2, \dots, t$); r_j denotes effect of the j^{th} replicate (superblock) ($j = 1, 2, \dots, r$); $B_{k(j)}$ denotes effect of the k^{th} incomplete block within the j^{th} replicate ($k = 1, 2, \dots, s$) and e_{ijk} denotes experimental error associated with the observation of the i^{th} treatment in the k^{th} incomplete block within the j^{th} complete replicate.

b. Estimation of Phenotypic and Genotypic Correlations

The phenotypic and genotypic correlation coefficients were calculated using Multivariate Restricted Maximum likelihood (REML) with Proc MIXED of the SAS system v.9.1 software.

$$\text{Phenotypic correlation coefficients (Pr)} = \frac{\text{Cov}_{p12}}{\sqrt{(\sigma^2_{p1}) \times (\sigma^2_{p2})}}$$

Where, Cov_{p12} is the phenotypic covariance of the progeny means between the two traits, and σ^2_{p1} and σ^2_{p2} are the phenotypic variance for each trait.

$$\text{Genotypic correlation coefficients (Gr)} = \frac{\text{Cov}_{g12}}{\sqrt{(\sigma^2_{g1}) \times (\sigma^2_{g2})}}$$

Where, Cov_{g12} is the genotypic covariance between two traits, σ^2_{g1} is the genotypic variance of the first trait, and σ^2_{g2} is the genotypic variance of the second trait.

3. RESULTS AND DISCUSSION

3.1. Soil Moisture Conditions

The soil water content was considerably reduced from flowering onward until physiological maturity in the stress trial [Figure 1](#). The intensity of drought increased with increase in time from which the stress was induced and is verified in terms of increment of tension force on the plant root to suck water. Variation in moisture content was recorded at different soil depths both under stress and non-stress conditions. The soil moisture depletion started at the top soil layer and moved gradually down to the deeper soil layer due to deep percolation and/or evaporation. The observed gradual soil moisture depletion trend across the soil depth significantly affected the potato plant that possesses a shallow root system. The shaded area is the range (60-90 KPa) whereby irrigation should be employed.

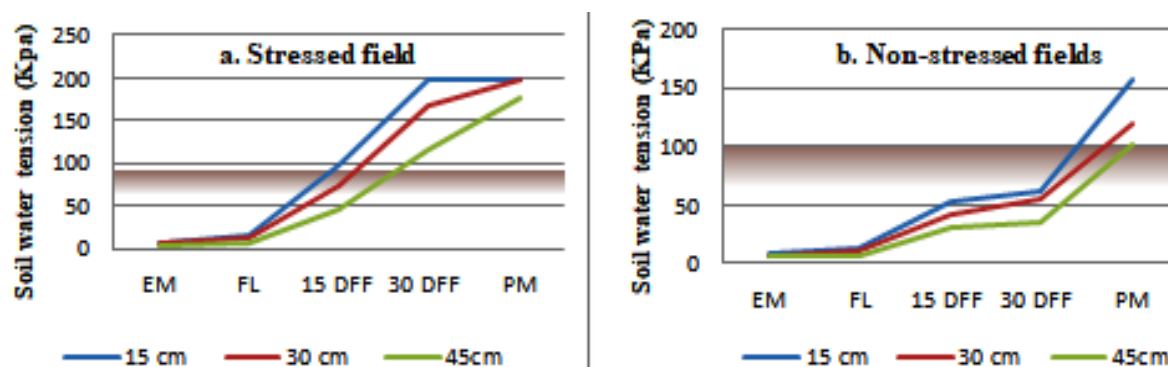


Figure-1. Soil moisture status of the stressed and non-stressed experimental sites.

3.2. Response of Leaf- Water Relation Traits for Drought Stress

Significant ($p \leq 0.05$) difference was observed between genotypes in morphological traits of potato such as Days to physiological maturity, plant height, above ground biomass and harvest index in both stressed and non-stressed environments Table 3. The detail genotypic variations of these traits were discussed below.

a. Above Ground Biomass

Compared to the non-stressed environment above ground biomass was declined by 6.3 % under stress environment. The ability of potato to form a large above ground biomass is an effective insurance against soil water deficit [7]. Biomass reduction of potato crops in response to drought is in agreement with a study by Saravia, et al. [8] who reported as drought significantly reduced the total biomass of potato. A small above ground dry weight is considered to be associated with poor drought resistance in potato [9]. However, large above ground biomass could raise the rate of water loss through transpiration. Schittenhelm, et al. [7] reported that a cultivar with a compact canopy easily showed a reduced radiation interception even with a small reduction of shoot dry weight. Hence, a genotype with minimum above ground biomass and however, compact canopy could able to tolerate drought through minimizing evaporative losses and increasing photosynthetic efficiency.

b. Harvest Index

Harvest index shows the extent of remobilization of photosynthates to tubers [10]. Identifying genotypes that use photosynthates for greater tuber expansion instead of above ground biomass will help in drought resistance selection. Deblonde and Ledent [11] suggested that moderate drought conditions did not influence the harvest index. However, our finding revealed that drought caused a reduction in harvest index by 10.34 % compared to the non-stressed environment. The extent of differences in harvest index is dependent on potato cultivars tested in stressed condition [12]. Maintaining a high harvest index in drought prone environments may contribute to realize high and stable tuber yield of potato [9].

c. Leaf Area and Specific Leaf Area

Leaf area ranged from 58.63 - 179.42 cm² and from 53.05 - 174.39 cm² under non stress and stressed environments, with mean leaf area of 90.26 cm² for non-stressed and 95.70 cm² for stressed treatments, respectively. Drought stress decreases leaf expansion rate, hinders the development of new leaves and results in senescence [13]. However, the minimal variation in leaf area among stressed and non-stressed treatments might be due to lower expansion rate of leaf at flowering and then after. Anyia and Herzog [14] reported as specific leaf area is amongst the traits to serve as index for drought resistance. In our experimentation, Specific leaf area ranged from 6.74 - 17.0 cm²/g under non stress environments whereas it was 6.2 -18.9 cm²/g under stressed environment. A reduction in specific leaf area is observed for legume crops under water stress condition [15] possibly signifying thicker leaves which help in leaf water conservation because of the low surface/volume ratio. Specific leaf area was reduced by 2.2 % under non-stress condition compared to the stressed treatment. Bogale, et al. [16] also reported as the specific leaf area of Ethiopia durum wheat genotypes was increased by 12.6% under water deficit relative to the non-stressed treatment.

d. Excised Leaf Water Loss

Excised leaf water loss estimates leaf transpiration rate by measuring the proportion of water loss through leaf cuticles and could be used for screening genotypes for drought resistance. It has shown a promising prospect in identifying drought resistant wheat genotypes [17]. It has ranged from 0.69-2.02 under non-stressed and from 0.4-2.74 under stressed environment. Compared to the non-stressed treatment, excised leaf water loss has a 10.68 % reduction under stressed environment.

e. The Leaf Relative Water Content

Relative water content shows. Relative water content indicates the balance between the water absorbed by the leaf tissue and transpiration rate, and used as a most meaningful index of water status in the plant and dehydration resistance. Drought significantly influenced and caused an average leaf relative water content reduction of 10.7 % under stress compared to the non-stress. Maralian, et al. [18] and Mensah, et al. [19] also noted as relative water content decreases from 82.4 - 69.7 % in potatoes and from 79.8 - 66.5% in sesame, respectively as irrigation supply decreases. Thought it was cultivar dependent, the leaf relative water content of potato declined markedly under stressed condition [20]. Relative water content of the genotypes ranged from 56-76% under stress treatment and from 74-96% under non-stress environments in the current study. Schonfeld, et al. [21] observed a decline in the amount of relative water content in wheat due to drought and reported the highest relative water content in the tolerant genotype.

f. Tuber Size Grade Distribution, Number and Weight

The larger size tubers percentage ranged from 1.74-7.84 % in non-stressed and from 0.7-6.5 % in stress treatment with a mean of 5.59 and 4.17 %, respectively. Medium size tubers ranged from 37.2-92.4 % in non-stressed and from 53.77-97.03% in stress environment with a mean of 62.23 and 74.47 %, respectively. Smaller tubers ranged from 0.76-2.8 % in non-stressed and from 0.8-4.07 % in stress environment with a mean of 1.41 and 1.67 %, respectively. Drought stress had a statistically significant influence on size distribution of potato tubers. The percentages of medium and smaller tubers were by 19.66 and 18.7 % higher under stress environment than the non-stressed condition. Gregory and Simmonds [22] reported a decline of 25.31 % of larger size tubers with exposure to drought. Mahmud, et al. [23] also reported a small proportion of smaller and medium sized tubers compared to the larger sized tuber under non-stress condition and the vice versa under drought conditions. The same author articulated as water deficit during tuber formation stage is a contributing factor for the decrease in number and size of potato tubers under drought condition. Drought caused about 33 % marketable and total tuber yield reduction compared to the non-stressed condition. Luitel, et al. [24] reported a 78.4 % reduction on marketable tuber yield due to the post-emergence stress as compared to well-irrigated condition. Similarly, Shi, et al. [20] reported 37-64 % tuber yield dropdown in potato cultivars exposed to stress compared with the non-stressed. Total tuber yield ranged from 12.34 to 45.1 tha⁻¹ under non-stress with mean of 31.9 tha⁻¹. Under stressed condition, total tuber yield ranged from 8.9 to 31.94 tha⁻¹ with mean of 21.35 tha⁻¹. Yield per plant ranged from 0.3-1.02 kg under non-stress and from 0.2-0.71 kg under stress treatment with mean tuber yield per plant of 0.72 and 0.48 kg, respectively. Drought reduced total tuber number per plant by 10.23 % on potato genotypes considered for this study. Similarly, Luitel, et al. [24] reported the total tuber number decrease of 7 % under drought compared to the non-stressed treatment.

3.3. Correlation of Leaf-Water Relation Traits with Tuber Yield of Potato

Better understanding of the relationship of various traits that affect the growth, productivity and quality of the potato crop is of greater importance in variety development as selection for one trait might cause an increase or deterioration on the other trait. Table 4 revealed as phenotypic and genotypic correlation exists between tuber yield and its contributing characters under both moisture regimes. In majority of the cases, the genotypic correlation coefficients were greater than the corresponding phenotypic correlation coefficients. The lower phenotypic correlation could be attributed to the environmental modification on the expression of the phenotypic correlations between traits. Johnson, et al. [25] also reported that the higher genotypic correlation than phenotypic correlation indicated an inherent association between various characters of soya bean.

Improvement of tuber yield in potato is possible by using appropriate breeding strategy through selecting for those traits which are positively associated with yield per plant. In this view, under stressed environment, total

tuber yield had a significant and positive genotypic correlation coefficients with harvest index ($r=0.996$), leaf area ($r=0.71$), specific leaf area ($r=0.69$). The positive association of tuber yield with harvest index is in agreement with the finding of Addisu, et al. [26]. Maintaining a high harvest index, leaf area and specific leaf area under drought prone environments, therefore, may significantly contribute to realize a high and stable potato yields which is in accordance with Deguchi, et al. [9] and Muchow [27]. On the contrary, the total tuber yield had a significant and negative association with above ground biomass ($r=-0.92$) and relative water content of leaf ($r=-0.51$). Hence, genotypes with higher above ground biomass and relative water content under stressed environments are susceptible for drought stress. The pattern of stomatal response under stress condition is an important factor in determining the sensitivity of a plant to water stress. Some plant species close their stomates to reduce water loss, while others leave their stomata open allowing photosynthesis to continue. This signifies that photosynthetic activities were continued even under stress condition with the expense of water loss from leaves. Under water stress condition, sorghum had higher yielding capability than maize due to the ability of sorghum to maintain stomatal aperture at lower water content Turner [28]. However, Schonfeld, et al. [21] reported the highest relative water content in the drought tolerant genotypes of wheat.

4. SUMMARY AND CONCLUSION

Water stress significantly influenced the expression of various leaf-water relation traits, and adversely affected tuber yield and quality attributes of the genotypes. The potato genotypes showed variable sensitivity to drought. The differential response of genotypes could be related with genetic architectural dissimilarities among genotypes and their effect on some leaf-water relation traits responses. Though the intensity of stress was considered as mild, post flowering drought caused 33.13% tuber yield reduction as compared to the non-stressed condition. Phenotypic and genotypic associations were detected between tuber yield and other traits which can potentially be employed for selection in breeding strategies. Under stress treatment, harvest index, leaf area and specific leaf area exhibited positive association with tuber yield, whereas above ground biomass and relative water content showed negative correlation with tuber yield. The traits which exhibited a higher correlation with yield of potato under stress condition could be used as a selection tool for future breeding works on drought resistance.

Table-3. Mean square of leaf-water relation traits and yield of potato genotypes, 2016.

Environment	Sources	AGB	HI	LA	SLA	ELWL	RWC	TY
Stressed condition	Group	1358.11 ^{ns}	0.00027 ^{ns}	8060.76 ^{**}	112.35 ^{**}	0.98 [*]	17.69 ^{ns}	0.60 ^{ns}
	Block (group)	1571.39 ^{ns}	0.013 [*]	404.7 [*]	4.2 ^{ns}	0.23 ^{ns}	77.14 ^{ns}	20.55 ^{ns}
	Entry	2231.57 ^{ns}	0.016 ^{**}	1072.27 ^{**}	9.36 [*]	0.35 ^{**}	85.08 [*]	46.09 [*]
	Error	1545.58	0.006	350.09	3.86	0.14	49.36	30.18
	CV (%)	28.72	12.4	19.55	19.57	31.63	9.36	25.73
Non-stressed condition	Group	20769.11 ^{**}	3.3x10 ^{-06ns}	1232.2 [*]	6.56 ^{ns}	4.85 ^{**}	24.60 ^{ns}	176.76 [*]
	Block (group)	2118.32 [*]	4.2x10 ^{-03*}	383.24 ^{ns}	2.97 ^{ns}	0.29 ^{ns}	40.89 ^{ns}	49.48 ^{ns}
	Entry	2886.71 ^{**}	1.1x10 ^{-02**}	694.27 ^{**}	5.51 [*]	0.34 [*]	60.02 ^{ns}	52.81 [*]
	Error	1012.02	1.7x10 ⁻⁰³	264.42	2.41	0.18	45.41	26.8
	CV (%)	21.77	6.03	18.02	15.8	32.22	8.01	16.21

Table-4. Genotypic (above diagonal) and phenotypic (below diagonal) correlations of traits in potato, 2016.

Traits	Non-stressed environment							Stressed environment						
	AGB	HI	LA	SLA	ELWL	RWC	TY	AGB	HI	LA	SLA	ELWL	RWC	TY
AGB		-0.79**	-0.16	-0.17**	-0.69**	-0.79**	-0.35**		-0.95**	-0.2	-0.31**	-0.50**	0.63**	-0.92**
HI	-0.51**		0.15	0.32**	0.50**	-0.17	0.86**	-0.53**		0.57**	0.63**	0.26**	-0.38**	0.996**
LA	-0.07	0.2		1.00**	0.12**	-0.56**	0.2	-0.031	0.34**		0.99**	-0.24*	-0.63**	0.71**
SLA	-0.07	0.24	0.99**		0.17**	1.00**	0.32**	-0.05	0.36**	0.97**		-0.26**	-0.71**	0.69**
ELWL	-0.37**	0.26*	0.001	-0.01		-0.32*	0.23**	-0.20	0.07	-0.03*	-0.03		-0.51	0.07
RWC	-0.10	0.06	-0.30*	0.36**	0.02		-0.73**	0.18	-0.11	-0.10	-0.09	-0.12		-0.51**
TOT	0.23	0.53**	0.17	0.19	-0.07	-0.02		-0.05	0.74**	0.4*	0.41**	-0.02	0.02	

CV: Coefficient of variation; AGB: above ground biomass, HI: harvest index, LA: leaf area, SLA, Specific leaf area, ELWL: excised leaf water loss, RWC: relative water content of the leaf; TOT: total tuber yield; * and ** represent significant differences at the 0.05 and 0.01 levels, respectively.

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