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Morphological and Physiological Measurement of the Stay-Green Trait in Transgenic and Non-Transgenic Cassava under Green-House Water Stress Conditions

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Abstract - Drought stress is the major abiotic factor that limits cassava productivity in many agro-ecological regions of sub-saharan Africa. In this study, stay-green trait in two transgenic cassava genotypes (transformed with *isopentenyl transferase (ipt)* gene for improved drought tolerance) and six non-transgenic cassava genotypes were evaluated under green-house conditions. Leaf abscission (for leaf retention), elongation of the last internodes, photosynthetic rates, and stomatal conductance were determined in these cassava genotypes subjected to three levels of water stress treatments (0, 30, and 60 %) and a positive control or fully irrigated plants. Two non transgenic genotypes (98-0002 and 98-2226) and one transgenic line (529-48) that expressed relatively high level of stay green or leaf retention, also exhibited significantly higher photosynthetic rates, internode elongation and relatively low stomatal conductance compared to other genotypes. Non transgenic genotypes 91-02322 and TME-3 and transgenic line 529-28, expressed moderate levels of stay green and non transgenic genotype 95-0306 and wild type TMS 60444 (for the transgenic lines) were highly susceptible to the water stress treatments. The results reported here showed there was a positive correlation between leaf retention, photosynthetic rates, internode elongation and stomatal conductance.

Keywords - Leaf Retention, *Manihot Esculenta* Crantz, Photosynthetic Rate, Stomatal Conductance, Transgenic and Non-Transgenic Cassava

1. Introduction

Cassava (*Manihot esculenta* Crantz), is ranked as the fourth most important food source for energy after rice, sugarcane and maize [1,2] The crop is mostly grown by small scale, resource-limited farmers on marginal and highly eroded low fertility soils without application of agrochemicals and in areas with uncertain rainfall patterns and prolonged dry periods [1].

Although cassava has a high growth rate under optimal conditions, the crop almost never fails to produce due to drought when compared to other staple crops [3]. The inherent ability of cassava to yield under stressful environments has enhanced its dominance as a food security crop in semi-arid agro-ecological zones.

Various morphological and physiological traits contribute to the ability of cassava to produce under difficult or marginal conditions. One such trait referred to as stay-green is the ability to retain leaves longer under stress and delay leaf senescence [4]. Drought stress causes leaf senescence, resulting in a reduced canopy size, lower photosynthesis rates and reduced yields [5]. It has been demonstrated that stay green genotypes are able to retain more green leaf area compared to genotypes without stay green trait during water deficits [3,4]. Staygreen has been characterized in certain cassava cultivars showing enhanced ability to retain their leaves under drought conditions. For example, [6] reported that when cassava was grown on very poor soils under prolonged drought for more than six months, the crop reduced its leaf canopy and transpirational water loss or stomatal conductance, but its attached leaves remained photosynthetically active, though at lower rates. When compared to clones without leaf retention trait, staygreen cassava clones produced more total fresh biomass, high fresh root yield and root dry matter and expressed high drought tolerance during water deficit [3,7]. By retaining their functional leaves with high water use efficiency and reducing the production of new leaves under drought conditions, drought tolerant or leaf retaining cassava cultivars are able to permit greater photosynthate accumulation in the roots and thus increase the harvest index [3]. Increased biomass production and the higher harvest index of cassava clones with leaf retention trait, when grown under prolonged period of drought, did result in a 32% greater av-

erage root yield when harvested towards the end of the dry season and 39% at the beginning of subsequent wet season [3,7].

Senescence, a type of cell death program inappropriately activated during stress enhances drought tolerance [5]. *In vitro* and greenhouse grown cassava lines transformed with the *isopentenyl transferase (ipt)* gene, from *Agrobacterium tumefaciens*, under the control of the *PSAG12* promoter, expressed significant stay-greenness or resistance to leaf senescence after drought treatment compared to wild-type plants [8].

The main objective in this study was therefore to evaluate the characteristics of the stay-green trait in transgenic cassava genotypes (transformed with *ipt* gene for improved reduction in leaf senescence) compared to those of non transgenic cassava genotypes under varying water stress treatments in the greenhouse. Consequently, the study investigated the relationship between leaf retention (stay green trait) and photosynthetic rates, stomatal conductance and internode growth among transgenic and non-transgenic genotypes.

2. Materials and Methods

2.1. *Cassava genotypes selected for study*

Two transgenic cassava lines (expressing ipt gene for improved leaf retention or reduced leaf senescence under water stress), a wild type and five non transgenic cassava genotypes were selected for establishment in the greenhouse. The two transgenic lines, 529-28 and 529-48 and their wild type TMS 60444 were obtained from ETH-Zurich, Institute of Plant Sciences, Plant Biotech. Lab. Library, Zürich, Switzerland. The non transgenic genotypes, selected from farmer fields, included 91-02322, 98-0002, 98-2226, TME-3 and 95-0306 provided by the International Institute of Tropical Agriculture (IITA, PMB, 5320, Ibadan, Oyo state, Nigeria).

2.2. *Multiplication, Growth and Establishment*

Cassava basic media (CBM) consisting of Murashige & Skooge salts with vitamins, 2 µM $CuSO_4$, 2% Sucrose, 0.3% gelrite, pH set at 5.8, and autoclaved for 1hr was formulated for multiplication. For establishment in the greenhouse the media or substrate used consisted of a 1:1 mixture of Soil (Topf und Pikiererde, 140, Ricoter, Aarberg, Switzerland) and Perlite (GS-Forma-SA, Mezzovico-Vira, Lugano, Switzerland). The soil component was a mixture of sand, compost, small pieces of plant debris and other organic materials.

The genotypes were *in vitro* multiplied for 45 days under growth chamber conditions set at a 12 hour light exposure and a constant temperature of 26°C (day/night) and later transferred to the greenhouse where they were transplanted to 0.45L pots (one plantlet per pot, filled with substrate) and hardened for 30 days. After hardening, the plants were transferred to substrate filled 1L pots (one plant per pot) where they were grown under irrigation for 135 days after which water stress treatments were commenced. Conditions for growth and establishment in the greenhouse were set at temperature of 17°C/26°C (day/night), 60%/50% (day/night) relative humidity (%RH), 14 hours of light at an intensity of 35 klux and an average air ventilation rate of 84.7%.

2.3. *Water stress treatments*

Three water stress treatments or levels (0, 30 and 60%) and a positive control were formulated based on a method for establishing different water deficit treatments as described by [9]. The water stress levels were based on percent water supplied to control plants.

The treatments included fully irrigated (100% irrigation) treatment (positive control), 60% irrigation, 30% irrigation and no-irrigation (0%) treatments. Zero percent treatment was attained by withholding total irrigation.

After 135 days after planting (DAP; here referred to as day 0), the plants were randomly assigned to the 4 treatments. Twelve plants of uniform growth (in stature) were selected from each genotype (96 plants in total). Each treatment was then assigned 3 plants (replicates) of each genotype (24 plants for each treatment). The genotypes were then submitted to the different water stress treatments for 20 days during which rates of photosynthesis, stomatal conductance, internode growth and leaf abscission (for leaf retention) were taken from each plant.

The genotypes and treatments were arranged in a randomized complete design (RCD) and the entire experiment was replicated three times.

2.4. *Measurement of parameters*

Measurement of leaf abscission, elongation of the last internodes, rates of photosynthesis and stomatal conductance were taken from Day 0 (135 DAP) and subsequently after every 2 days for the entire period of water stress treatments. The measurements were taken between 9 am and 2 pm.

2.5. *Leaf Abscission (LA) for leaf retention*

The highest leaf scar or petiole (from the soil level) and the oldest folded leaf scar or the last fully expanded leaf (top most) was tagged and the total number of leaves in between counted and recorded from each plant.

The tagged leaf scar close to the soil level was henceforth used as a baseline and leaf retention was determined by counting the number of leaves abscising above this leaf scar. The new highest leaf scar was subsequently tagged upon recording a new leaf drop. The number of leaves dropped was then counted and recorded.

2.6. *Elongation of the Last Internode (ELI)*

The base petiole of the last growing internodes from each plant was tagged and the initial internode length measured and recorded using a centimeter rule. Subsequent internode lengths were measured after every 2 days and the internode elongation was determined by subtracting the new length from the previous length. The original lengths were used as covariates during data analysis.

2.7. *Rate of Photosynthesis (PN) and Stomatal Conductance (Gs)*

Three last fully expanded or unfolded leaves (top most), and one leaf at mid-stem of each plant were tagged and P_N (umolCO2 m^{-2} s^{-1}) and Gs (mmol m^{-2} s^{-1}) were measured from these four leaves using an Infrared Gas Analyzer (IRGA) equipped with a modulation Fluorometer (LI-COR 6400 Photosynthesis System, Lincoln, NE, USA) following manufacturer's instructions.

2.8. *Data Analysis*

All the data collected for each parameter was subjected to analysis of variance (ANOVA) using the PROC ANOVA procedure of Genstat Discovery Edition 3 (Lawes Agricultural Trust Rothamsted Experimental station, UK). The differences among the treatment means were compared or separated using the Fisher's protected Lsd test at 5% probability level.

Correlation analyses among the parameters were also done using the same program. The four P_N and Gs values for each day were pooled by calculating their averages which were then used in the subsequent analysis of variances.

3. Results

3.1. *Effect of water stress treatment on leaf retention, internode growth, photosynthetic rates and stomatal conductance*

Significant differences in internode growth, leaf abscission, photosynthetic rates and stomatal conductance were observed within water stress treatments. Both fully irrigated (control) cassava genotypes exhibited significantly (P≤0.05) higher leaf retention, internode elongation, photosynthetic rates and stomatal conductance compared to cassava genotypes submitted to other water stress treatments (Tables 1, 2, 3 and 4).

The non-irrigated genotypes consistently expressed lower (significant at P≤0.05) leaf retention, internode growth, photosynthetic rates and stomatal conductance than genotypes subjected to 30% and 60% irrigation as well as control plants. Cassava genotypes subjected to 60% irrigation treatment expressed significantly (P≤0.05) higher leaf retention, internode increment and photosynthetic rates than genotypes under 30% water deficit. No significant (P>0.05) variation was observed in amount of water vapor conducted by cassava genotypes subjected to 30% and 60% irrigation treatment (Tables 1, 2, 3 and 4).

3.2. *Internode growth variation in transgenic and non transgenic cassava genotypes subjected to water stress treatment under greenhouse conditions*

Significant variation in internode elongation was observed in transgenic cassava lines 529-28, 529-48 and their wild type TMS 60444. Internode of line 529-48 lengthened significantly (P≤0.05) more than the internode of line 529-28 and wild type TMS 60444. Inter-node of line 529-28 showed the least significant (P≤0.05) increment (Table 1).

When internode growth was compared between transgenic and non transgenic cassava genotypes, the internode of transgenic line 529-48 lengthened significantly (P≤0.05) more than the internodes of non-transgenic genotypes. All non transgenic cassava genotypes (except 95-0306) significantly (P≤0.05) showed higher internode increment than internode of transgenic line 529-28. There was no significant (P>0.05) internode growth variation between line 529-28 and genotype 95-0306. Significant differences in internodal growth were also observed between wild type TMS 60444 and non transgenic genotypes (Table 1).

Table 1. Mean internode growth or elongation (in cm) of cassava genotypes subjected to different levels of water stress regimes under greenhouse conditions.

	Treatments				
Genotype	0% Irrigated	30% Irrigated	60% Irrigated	Fully Irrigated	Mean
529-28	0.16i	0.34n	0.38t	0.56s	0.36e*
529-48	0.29fg	0.56j	0.65q	0.78u	0.57a
TMS 60444	0.20hi	0.40m	0.56r	0.70v	0.47c
91-02322	0.21h	0.37mn	0.48s	0.60g	0.41d
98-0002	0.17i	0.37mn	0.51s	0.73v	0.44c
98-2226	0.27g	0.47k	0.59r	0.73v	0.52b
95-0306	0.15i	0.26p	0.36t	0.51o	0.32e
TME-3	0.31f	0.39m	0.49s	0.65i	0.46c
Mean (Treat)	0.22z	0.40y	0.50x	0.66w	

Lsd (P≤0.05) Treat = 0.03 Lsd (P≤0.05) Genotype = 0.04

* Means followed by the same letter(s) are not significant from each other

3.3. *Leaf Abscission (for leaf retention) variation in transgenic and non transgenic cassava genotypes subjected to water stress treatment under greenhouse conditions*

No significant differences (P>0.05) in total number of leaves dropped by line 529-28 and wild type TMS 60444 were observed, indicating a similar level of leaf retention between the two genotypes. Transgenic line 529-48 lost significantly (P>0.05) fewer leaves than line 529-28 and wild type TMS 60444 and consequently expressing higher leaf retention or stay-greenness than line 529-28 and wild type TMS 60444 (Table 2).

Table 2. Mean total number leaf abscission (for leaf retention) from cassava genotypes subjected to water stress treatment under greenhouse conditions.

	Treatments				
Genotype	0% Irrigated	30% Irrigated	60% Irrigated	Fully Irrigated	Mean
529-28	1.89p	1.81m	1.47b	0.01g	1.29a*
529-48	1.54u	1.40n	1.14i	0.02g	1.03bd
TMS 60444	1.83pr	1.93m	1.57b	0.09g	1.36a
91-02322	1.62su	1.33nk	1.24hi	0.06g	1.06bd
98-0002	1.54u	1.21k	0.92j	0.04g	0.93cd
98-2226	1.56tu	1.44n	1.16h	0.05g	1.05bd
95-0306	1.79pr	1.87m	1.60b	0.03g	1.32a
TME-3	1.72qrs	1.47n	1.30h	0.03g	1.12b
Mean (Treat)	1.69w	1.56x	1.30y	0.04z	

Lsd (P≤0.05) Treat = 0.10 Lsd (P≤0.05) Genotype = 0.15

* Means followed by the same letter(s) are not significant from each other

Genotype 95-0306 lost significantly (P≤0.05) more leaves (lower leaf retention) than other non transgenic cassava genotypes. Although the total number of leaves shed by genotypes 98-0002, 98-2226 and 91-02322 were not significantly (P>0.05) different, trends indicated lower leaf abscission (high leaf retention) in 98-0002, followed by 98-2226 and 91-02322 respectively. Leaf retention in genotype TME-3 was not significantly different (P>0.05) from leaf retention in genotype 98-2226 and 91-02322, although TME-3 expressed significantly lower leaf retention than 98-0002 (Table 2).

Variations in leaf retention were also observed when transgenic cassava lines, wild type and non-transgenic cassava genotypes were compared. Although non transgenic genotypes 98-0002, 98-2226, 91-02322 and transgenic line 529-48 expressed relatively similar (P>0.05) levels of leaf retention, the general trends showed a descending leaf retention from genotype 98-0002, transgenic line 529-48, 98-2226 and 91-02322 respectively. Leaf retention in line 529-28, wild type TMS 60444 and genotype 95-0306 were relatively similar (P>0.05), but significantly (P≤0.05) lower than leaf retention in genotype TME-3. Transgenic line 529-48 and genotype 98-0002 dropped the least total number of leaves i.e. expressed the highest leaf retention (Table 2).

3.4. *Photosynthetic rate (PN) variation in transgenic and non transgenic cassava genotypes subjected to water stress treatment under greenhouse conditions*

Transgenic line 529-48 expressed significantly (P≤0.05) higher P_N than wild type TMS 60444 and line 529-28. Although there was no significant difference (P>0.05) in P_N between wild type TMS 60444 and line 529-28, observed trend showed wild type TMS 60444 with higher mean P_N (Table 3).

Among non transgenic genotypes, the rates of photosynthesis in genotype 98-0002 and 98-2226 did not significantly (P>0.05) differ, although the two genotypes expressed higher P_N when compared to other non transgenic genotypes (Table 3). No significant (P>0.05) variation was observed between P_N of genotypes TME-3 and 91-02322, as well as between the P_N of genotypes TME-3 and 95-0306. Genotype 91-02322 expressed significantly (P≤0.05) higher P_N than genotype 95-0306 (Table 3).

Comparing transgenic and non-transgenic cassava genotypes also revealed significant variation in rates of photosynthesis. Although the P_N in transgenic line 529-48, genotypes 98-0002 and 98-2226 did not significantly (P>0.05) differ, observed trends showed line 529-48 with higher mean P_N than 98-0002 and 98-2226. Rates of photosynthesis in genotype TME-3, wild type TMS 60444, transgenic line 529-28, and genotype 95-0306 were not significantly (P>0.05) different, although the most and least P_N means were observed in genotype TME-3 and 95-0306 respectively. Genotype 91-02322 expressed significantly (P≤0.05) higher and lower P_N than transgenic line 529-28 and 529-48 respectively (Table 3)

Table 3. Mean rates of photosynthesis (PN; umolCO2 m-2 s-1) of cassava genotypes submitted to water stress regimes under greenhouse conditions.

	Treatments				
Genotype	0%Irrigated	30% Irrigated	60% Irrigated	Fully-Irrigated	Mean
529-28	2.13p	4.09wy	4.40iz	6.10tv	4.18d*
529-48	3.01m	5.42r	5.84e	8.27p	5.64a
TMS 60444	2.56no	4.30vxy	4.59hz	6.29t	4.43bd
91-02322	3.00m	4.37uvxy	4.88fh	7.04s	4.82b
98-0002	3.90k	5.12rt	6.20e	7.12rs	5.58a
98-2226	3.10m	4.73stv	5.89e	8.21p	5.48a
95-0306	2.68mo	4.05y	4.63ghz	5.74uv	4.28cd
TME-3	2.76mo	4.70tx	5.10f	7.74q	4.43bd
Mean (Treat)	2.89z	4.60y	5.19x	7.06w	

Lsd (P≤0.05) Treat = 0.31 Lsd (P≤0.05) Genotype = 0.44

* Means followed by the same letter(s) are not significant from each other

3.5. *Variations in stomatal conductance (Gs) of transgenic and non transgenic cassava genotypes subjected to water stress treatment under greenhouse conditions*

Although Gs in transgenic lines 529-28, 529-48 and wild type TMS 60444 were not significantly (P>0.05) different, the mean Gs values were higher and lower in wild type TMS 60444 and line 529-48 respectively. Among non transgenic genotypes, Gs did not significantly (P>0.05) vary in genotypes 98-0002, 95-0306 and TME-3. Similar results were observed between genotypes 98-2226 and 91-02322.

Despite these observations, trends indicated higher Gs mean in genotypes 98-0002 and 95-0306 and lower Gs means in genotypes TME-3, 91-02322 and 98-2226 (Table 4).

When Gs was compared between transgenic and non-transgenic cassava genotypes, Gs in transgenic lines 529-28, 529-48 and wild type TMS 60444 did not significantly vary (P>0.05) vary with the Gs of non transgenic genotypes 98-0002, 95-0306 and TME-3. The Gs of genotype 98-2226 and 91-02322 were significantly lower (P≤0.05) than Gs of the transgenic lines 529-28, 529-48 and wild type TMS 60444 (Table 4).

Table 4. Mean Stomatal Conductance (Gs; mmol m–2 s–1) of cassava genotypes submitted to water stress treatments under greenhouse conditions.

	Treatments				
Genotype	0% Irrigated	30% Irrigated	60% Irrigated	Fully Irrigated	Mean
529-28	0.025deg	0.052h	0.043qv	0.051jk	0.043a*
529-48	0.021fg	0.046ijm	0.047q	0.053hik	0.042ad
TMS 60444	0.035a	0.049hj	0.046qs	0.053hik	0.046a
91-02322	0.029bce	0.039np	0.037tw	0.042m	0.037cd
98-0002	0.029bce	0.052h	0.043qv	0.060g	0.046a
98-2226	0.025deg	0.037p	0.044qu	0.051jk	0.039bd
95-0306	0.032ac	0.050hj	0.044qu	0.057gi	0.046a
TME-3	0.029bce	0.041kmp	0.041rsuvw	0.057gi	0.042ad
Mean (Treat)	0.028y	0.046x	0.043x	0.053w	

Lsd (P≤0.05) Treat = 0.004 Lsd (P≤0.05) Genotype = 0.006

* Means followed by the same letter(s) are not significant from each other

3.6. *Correlations coefficients of photosynthetic rates (PN), stomatal conductance (Gs) and leaf abscission (LA)*

The positive correlation between P_N and Gs, negative correlation between P_N and LA and the negative correlation between LA and Gs were all significant at P≤ 0.01 (Table 5).

Table 5. Correlation coefficients of parameters; Stomatal Conductance, Leaf Abscission and Rates of Photosynthesis of water stressed cassava genotypes

Trait	Stomatal conductance (Gs)	Leaf Abscission (LA)	Photosynthetic rate (P_N)
Gs	1	-0.099 *	+0.476 *
LA		1	-0.252 *
P_N			1

*Correlation is significant at the (P≤ 0.01).

Genotypic variations in correlations between the above stated parameters were observed. For example, line 529-48, genotypes 98-0002 and 98-2226 expressed high P_N low LA compared to line 529-28, wild type TMS 60444, and genotype 95-0306 that expressed high LA and low P_N (Tables 2 and 3). The negative correlation between P_N and LA (Table 5) were also consistent in genotypes 91-02322 and TME-3 (Tables 2 & 3). Although Gs of transgenic genotypes 529-28, 529-48 and wild type TMS 60444 were not significantly (P>0.05) different, line 529-48 still exhibited both high P_N and Gs. Non transgenic genotype 98-0002 also expressed high P_N and high Gs compared to other genotypes (Table 3 and 4).

4. Discussion

This study investigated and revealed that water stress treatment under greenhouse conditions generated significant variations in rates of CO_2 uptake (photosynthesis), amount of water vapor conducted (stomatal conductance), leaf abscission (leaf retention) and elongation of last internodes (internodal growth) among transgenic and non transgenic cassava genotypes.

With regard to specific water stress treatment, the results showed that fully irrigated (control) plants expressed significantly higher mean values of leaf retention, internode growth, rates of photosynthesis and stomatal conductance compared to performance of cassava plants subjected to 0%, 30% and 60% water stress treatment as would be expected. The plants where there was no irrigation at all expressed the least values of these parameters as would be expected. Cassava plants that were subjected to 60% irrigation expressed higher photosynthetic rates, internode increment and leaf retention than those subjected to 30% irrigation. The only exception was observed in stomatal conductance which did not significantly vary in plants under both sets of water stress treatments (30% and 60% irrigation).

Genotypic variations in internode growth in both transgenic and non transgenic cassava genotypes were identified in this study. For example internode of line 529-48 and genotype 98-2226 lengthened more compared to other genotypes, while wild type TMS 60444, line 529-28 and genotype 95-0306 elongated the least. Internode of genotype TME-3, 91-02322 and 98-0002 increased moderately. Although internode growth or increment has not been used to characterize staygreen in cassava under drought, other related growth parameters such as root length, shoot or canopy growth, plant height and stem girth has been reported to be water stress and genotype dependent [10,11].

In this study, leaf retention was found to be genotype dependent. For example the high leaf retaining genotypes included 98-0002, line 529-48, 98-2226 and 91-02322, while genotype 95-0306, line 529-28, and TMS 60444 showed low leaf retention.

Genotype TME-3 expressed moderate leaf retention. The variation in leaf retention ability of cassava genotypes as shown in the study, substantiate early research which indicated that leaf retention in cassava under drought stress is genotype dependent [9,10].

Cassava genotypic variation in photosynthetic rates (P_N) under drought stress as observed in this study, have also been previously reported [12].

In this experiment, the rates of photosynthesis was high in line 529-48, 98-0002 and 98-2226, moderate in genotypes 91-02322, TMS 60444 and TME-3 and lower in line 529-28. These variations can be attributed to the differences in cassava's stomatal sensitivity to lowered water status [12].

The amount of water conducted (stomatal conductance) under water stress also varied with genotypes. This genotypic difference was previously documented [10,11]. Although the mean stomatal conductance values were not significant in most genotypes, relatively similar but high amount of water vapor was conducted by transgenic lines 529-28, 529-48, wild type TMS 60444, genotypes 98-0002, 95-0306 and TME-3 compared to low stomatal conductance values of genotypes 91-02322 and TME-3.

Cassava genotypic variations in Gs under drought treatment can be attributed to differences in some inherent and environmental factors such as vapor pressure differences, leaf temperature, air velocity, leaf water potential, leaf water conductance, transpiration rates, stomatal size, distribution, opening or closure, and leaf area expansion.

High transpiration rates have been associated with high

stomatal conductance in cassava [13]. Leaf area expansion or growth in cassava decreases upon imposition of water deficit [14]. This limits expansion and development of transpirational surface area during water deficit [9] and thus affecting stomatal conductance. In addition, reviews have shown that under greenhouse conditions, the stomata may close or open depending on the cultivar or wind velocity [14].

There were positive correlation between photosynthetic rates and stomatal conductance, negative correlation between photosynthetic rates and leaf abscission as well as the negative correlation between leaf abscission and stomatal conductance. Similar results have been reported [13]. In non transgenic plants, genotype 98-0002 abscised fewer leaves, conducted more amount of water vapor and exhibited higher photosynthetic rates compared to other non transgenic genotypes. Genotype 95-0306 expressed high leaf abscission, high stomatal conductance and low photosynthetic rates. Similarly genotype 98-2226 expressed relatively low leaf abscission, conducted low amount of water vapor, and high photosynthetic rates.

Based on these correlation matrices, water stress ultimately affects leaf retention, rates of photosynthesis, and stomatal conductance. Similar correlation as observed in these parameters has been reported before [7,11].

5. Conclusions

The application of water stress levels at 30% and 60% of full irrigation showed that cassava genotypic variation in terms of their photosynthetic rates, leaf retention, and internode growth were detectable. Stomatal conductance is not a favorable parameter to characterize the stay-green trait. Non transgenic genotypes 98-0002, 98-2226, 91-02322, TME-3 and 95-0306 expressed high rates of photosynthesis in descending trend respectively, while photosynthetic rates in transgenic lines 529-48, wild type TMS 604444, and line 529-28 reduced respectively. The transformation of cassava with *isopentenyl transferase (ipt)* gene did not confer any advantages to the stay-green characteristics.

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