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Effect of seasonal variability on the physiological performance of selected cocoa (*Theobroma cacao* L) clones in Nigeria

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Abstract

Eleven cocoa clones namely AMAZ 15, ICS 95, IMC 47, MAN 15, PA 150, SCA 9, SPEC 54, UF 67, F3 AMAZON, N 38 and TC 2were hand pollinated and the seeds were raised for 6 months before being transplanted to the field at 6 months. Survival count of the clones was taken for 36 months starting from the first month of establishment. Data on the gas exchange characteristics of cocoa clones started when cocoa were 12 months and continued for the next 2 wet and 2 dry seasons. Majority of cocoa clones showed higher values of stomatal conductance, stomatal transpiration, photosynthetic rates, relative water content, cuticular transpiration and stomata density during wet season while eight of the eleven cocoa clones had higher water use efficiency performance in dry season. The regression analysis also showed a linear, positive and significant relationship between stomatal transpiration and stomatal density of all cocoa clones. The results showed significant differences in the abilities of cocoa clones to cope with water stress while majority of cocoa clones significantly differed between dry and wet seasons.

Keyword: Cocoa; Clones; Water stress; Water use efficiency

1. Introduction

Theobroma cacao L. is from a family of Malvaceea [1] and one of the most economically important cash crops in many tropical countries, where it is cultivated by nearly 6 million farmers [2]. Cocoa is the name used for both the tree and fruits of *Theobroma cacao* L. in Nigeria [3]. In 2021/2022 crop year, West Africa produced 3,589,000 tons of dry cocoa beans representing 74% of the global production of 4,826,000 tons while Nigeria with a production of 280,000 tons of dry cocoa beans and about 5.8% of the total global production in 2021/2022 season was the 5th largest exporter of cocoa beans behind Cote d'Ivoire, Ghana, Ecuador and Cameroun, in that order [4].

Being a tropical woody crop, species, cocoa grows as an understory of rain forests and grows in areas with high annual rainfall of between 1,500 and 2,000 mm/year [5]. Both the amount and distribution of rainfall are some of the most important environmental factors that affect cocoa growth and development [6, 7] as the production of this crop is prone to periodic drought due to seasonal rainfall patterns that most times are characterised by a long dry season that is becoming more unpredictable in the current climate changes scenario. Cocoa performs better in a moderate shade, especially during the first three years of its establishment as the young cocoa trees are prone to water stress during their early growth and development [8].Under water stress and high temperature conditions, cocoa show some

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adaptations at various physiological levels for their survival and growth, depending on their genes expression. Although, drought negatively impacts on the survival and growth of cocoa in Nigeria [9], the crop is still majorly grown as a rain fed crop and not much research has been directed towards the identification and development of drought tolerant cocoa germplasm in the country. Nigeria famers are currently faced with the problem of establishment new cocoa farms which is becoming more difficult during the first two dry seasons or first two years after transplanting to the field. This situation calls for more insight into the physiological and morphological responses of cocoa trees to water stress. For instance, the relationship between water availability and photosynthesis in cocoa should be known while basic knowledge of many physiological processes and responses in terms of the whole-tree-scale effects of water stress in cocoa is also highly essential[10]. These will lead to more practical and feasible field scale methods that will be more relevant to the management of water stress [11] which can be applied towards the breeding and agronomic research of cocoa. Meanwhile, since most research work on how cocoa reacts to stress are carried out under artificial stress conditions in the laboratories and greenhouses, the results may not be the representative of the crop response under typical field conditions [12]. There is a need for more on-site field research works that will be designed for the improvement of crop-soil water management and get basic understanding of the effects of water stress on plants [13]. Consequently, this study was carried out to evaluate the ecophysiology of eleven cocoa clones under same climatic and soil conditions during dry and wet seasons to identify their possible drought tolerant abilities in Nigeria.

2. Materials and methods

Eleven cocoa clones (AMAZ 15, ICS 95, IMC 47, MAN 15, PA 150, SCA 9, SPEC 54, UF 67, F3 AMAZON, N 38 and TC 2) from the Cocoa Germplasm Plot of Cocoa Research Institute of Nigeria (CRIN) Ibadan were selected for this work. Two of the clones, F3 Amazon and TC 2, had been previously released by CRIN to Nigerian farmers for commercial production. The pods from each clone were produced through hand pollination in order to get a true-to-type cocoa seeds. The seeds were raised as seedlings for 6 month in the nursery before being planted out at the experimental site located at CRIN, Ibadan, Nigeria (Latitude 7.26°, Longitude 3.54° and 122m above sea level). The experimental field was established with 11 cocoa clones, 10 stands of seedlings per clone and replicated 4 times to make a total of 440 experimental plants. The seedlings were planted out at 3 m by 3 m spacing and laid out in a complete randomised block design on a land which had already been established with plantain at 3 m by 3 m a month before cocoa establishment to serve as a nurse crop to the young cocoa plants. All the good agricultural practices for maintenance of cocoa farms were carried out. Manual weeding through slashing with cutlass was carried out four times per year on the plot while emerging pests and diseases were controlled with relevant CRIN-recommended agrochemicals and methodologies to prevent any infestation and/or infection from reaching its critical threshold on the cocoa plants. The soil samples of the experimental field were collected with the use of soil auger. Soil samples were taken from the top layer from 0-15 cm of soil collected at 14 different spots on the field and bulked together before being air-dried under room temperature for 3 weeks and then bulked together. A sample of the air-dried bulked soil was taken and kept in a sealed coded zip lock bag before being sent to an internationally accredited laboratory for soil analysis.



Figure 1 Infra-Red Gas Analyser (IRGA) being used in Ibadan to collect photosynthetic rate, stomatal conductance and stomatal transpiration at ≥300 µmol m⁻²s⁻¹ of sunshine needed for optimum performance of cocoa

Five stands of each cocoa clone were selected per replicate for sampling resulting into 20 stands per clone per site and data collection took place for two dry seasons and two wet seasons between year 2020 and 2022.Data on the cocoa survival count (recorded at the end of each dry season and wet season, photosynthetic rate, stomatal conductance (g_s) , stomatal transpiration, leaf relative water content, cuticular transpiration and stomatal density and water use efficiency were determined. The photosynthetic rates, stomatal conductance and stomatal transpiration were in collected in-situ with Infra-Red Gas Analyser (Fig. 1) while leaf relative water content (LRWC) and cuticular transpiration(CT) were determined in the laboratory. The stomatal density was determined both in-situ and laboratory.

The LRWC were measured when leaves of approximately the same physiological age were detached from the plants, mopped of any moisture molecules and immediately covered with black polythene nylon bags. The leaves were taken to the laboratory where 15 discs of 1.5cm diameter each were punched out from every sampled leaf. Leaf discs were immediately transferred into tagged covered petri dishes and weighed to determine their fresh weight (FW). About 40ml of distilled water was added to leaf discs in each petri-dish, covered and left to float for 24hrs at room temperature to allow them reach full turgor. Leaf discs were extracted and the water removed from the petri dishes. Both the leaf discs as well as the petri dishes were dried of surface water using filter papers to adequately mop them up. The leaf discs were transferred into clean brown envelopes before being dried in the oven at 65° C for 3hrs. The dried leaf discs were returned into the petri dishes and finally weighed again to determine their dry weights (DW). The leaf relative water content (RWC) of each cacao plant was then calculated using the method of Turner [14] as RWC = (FW-DW)/ (TW-DW) x 100%.

For CT determination, the method of Ayegboyin [3] was adopted. The 2nd or 3rd hardened apical leaf with its petiole was detached from the plants and immediately covered with black polythene nylon. Then the samples were taken to the laboratory where their fresh weights were determined with a top loading Analytical Balance 210g x 0.0001gm 50Hz/1pa after their petioles have been re-cut inside clean water. The petioles of the leaves were immediately dipped into distilled water, covered with black nylon and allowed to hydrate overnight at room temperature. After about 22 - 23 hours, the leaves were removed from the water, mopped of any moisture molecules on them and weighed again to determine their turgid weights. The samples were then placed in a Gallenkamp Cooled Illuminated Incubator 0-50°C/50Hz/1PH 80L capacity at 25 °C for 8 hours and leaves weights were recorded at 30 minutes interval. New weights are subtracted from the previous to get the amount of moisture loss per leaf in 30 minutes. The cuticular transpiration, which is quite less than stomatal transpiration in a typical cocoa plant takes place on the adaxial as well as the abaxial surfaces of cocoa leaves and was calculated as the slope of water loss from the leaves in mmol m⁻²s⁻¹.

For stomata density measurement on cocoa leaves, the procedures below were followed:

- Fully mature leaves to be sampled were tagged and labelled.
- Each of the leaves was painted with clear fingernail polish on the abaxial surface without its midrib. If the leaf surface was wet, it was first mopped with thick filter paper before painting. The painted spot was approximately 2cm wide and 4cm long.
- The fingernail polish was allowed to dry completely on the leaf for 3 to 5 minutes.
- A short strip of clear Scotch tape (not frosted) of approximately 5cm long was firmly pressed over the dried nail polish on the lower epidermis. The cello tape was carefully peeled from the leaf, pulling of the dried polish and gently affixed to a clean microscope slide.
- Each of the prepared slides was then place into a labelled brown envelope.
- The prepared slides were observed under an Axioscope 2 microscope with an Axiocam camera attached (*Carl Zeiss*, Jena, Germany) at 40X magnification. Image were saved from 3 different fields for each slide, stored with a memory stick and then transferred into computer.
- The number of stomata openings in the JPEG image was counted with the aid of ImageJ (Java-based image processing programme).

2.1. Statistical analysis

Data collected were subjected to Analysis of Variance before significant means were determined by least significant difference (LSD) at P = 0.05 value.

3. Results and Discussion

3.1. The results of the physicochemical analysis of the experimental soil is shown in Table 1.

Table 1 Result of Laboratory Soil Analysis of Experimental Site

	Parameter	Unit	Value
	рН		6.9
Particle size	Sand	%	36.4
	Silt	%	12.36
	Clay	%	51.6
Exchangeable bases	Са	cmol/kg	5.36
	Mg	cmol/kg	1.08
	К	cmol/kg	0.364
	Na	cmol/kg	0.494
	Al+H	cmol/kg	0.07
	ECEC	cmol/kg	7.37
Base	Salinity	%	99.05
Total	Ν	%	0.149
Organic	С	%	2.49
Available	Р	mg/kg	10.79
Micro nutrients	Mn	mg/kg	66.3
	Fe	mg/kg	1.35
	Cu	mg/kg	6.47
	Zn	mg/kg	12.75

The soil analyses showed that the experimental soil has a pH of 6.9 and ideal for cocoa production. Cocoa grows well in soils with a pH in the range of 5.0 - 7.5. Also, the 2.49 % level of organic matter revealed that the experimental soil was good and far above its critical threshold needed for crop growth [15]. The 0.15 % of total N in the sampled soil was considered optimal for cocoa [16] while 10.79 m gkg⁻¹of available P was higher than 10m gkg⁻¹ P regarded as minimum required quantity for crop production [15]. The distribution of particle sizes of Sand (36.04%), Silt (12.36%) and Clay (51.60%) showed that the sampled soil was clayey-loam with a fairly good water draining ability that is required for cocoa production. Majority of the areas under cocoa cultivation in Nigeria are either clayey-loam or sandy-loam soils. Beside the values of 5.36 cmol/kg, 1.08 kg and 0.494 cmol/kg for Ca, Mg, K and Na respectively showed that cmol/kg, 0.364 cmol/the exchangeable K, Ca, Mg and Na were higher than 0.2 cmol/kg regarded as the critical levels for each of these nutrients for optimum cocoa growth [17]. The 0.07 cmol/kg exchangeable Al+H showed a very low exchangeable Aluminum hydride. However, hydrated Aluminum species (Al combined with hydroxyl [OH-]) usually are not toxic to cocoa because their charge is too weak to displace basic cations (Ca²⁺, Mg²⁺) from soil exchange sites. As soil pH becomes lower, the decreasing soil pH will provide increasing H+ ion activity, which reacts with OH- ions and combines with the Al³⁺ ion, stripping the OH- away from the Al³⁺ and thereby increases the charge on the Al-species to a +2 or +3 charge.

3.2. Survival count

The record of plants survival was taken for 3 wet seasons and 3 dry seasons to determine how seasonal variations affect the survival of cocoa clones within the first 3 years of establishment (Table 2).

Cocoa clones	Season After Transplanting							
	1 st Wet SAT	1st Dry SAT	2 nd Wet SAT	2 nd Dry SAT	3 rd Wet SAT	3 rd Dry SAT		
AMAZ 15	40	39	37	37	37	37		
ICS 95	40	39	39	38	38	38		
IMC 47	40	40	39	38	38	38		
MAN 15	40	38	38	38	38	38		
PA 150	40	40	40	39	39	39		
SCA 9	40	40	40	40	40	40		
SPEC 54	40	40	40	39	39	39		
UF 67	40	37	37	37	37	37		
F3 AMAZON	40	40	40	40	40	40		
N 38	40	39	38	38	38	38		
TC 2	40	40	40	39	39	39		
P = 0.05	NS	NS	S	S	NS	NS		

Table 2 Survival count of cocoa clone during the first 3 years of establishment

Legends: SAT = Season after transplanting; NS = Not significant; S = Significant

The cocoa clones showed significant variability (P = 0.05) in the survival counts of cocoa based on individual clones and seasonal performances. While SCA 9 and F3 Amazon maintained perfect record throughout the data collection period. AMAZ 15 and UF 67 jointly had the lowest survival rate. It was observed that survival of cocoa plants become stable after the 2nd dry season which is about 24 months after establishment. This indicate that artificial watering of cocoa to supplement the regular rainfall in the first 24 months after establishment might have increased the overall survival ability of coca clones.

3.3. Stomatal Transpiration of cocoa clones



Figure 2 The stomatal transpiration rate of the fully expanded leaf of cocoa clones during dry and wet seasons. Values represent the means of leaves measured repeated on two occasions. Bars shows the Least Significant Differences

There were significant (P = 0.05) variation in the stomatal transpiration performance between clones and seasons while all clones recorded higher values during wet seasons with the exception of IMC 47 (Fig. 2). The 3.301 mmol $m^{-2}s^{-1}$

produced by F3 Amazon during wet season was significantly higher (P = 0.05) than those of IMC 47 and UF 67 at the same period of time while 3.134 mmol m⁻²s⁻¹ produced by same F3 Amazon in dry season was also significantly higher than those of N 38 and UF 67 at the same season. The performance of PA 150, SCA 9, F3 Amazon and TC 2 were not significantly different (P = 0.05) between clones within seasons. This shows that all cocoa clones continue to lose water throughout the year, irrespective of the seasonal availability of rainfall. Although, cocoa showed relatively lower transpiration rates during dry season, there was no 'zero value' even in peak of dry season and this revealed that cocoa might not easily stop stomatal transpiration even in dry season, and can explain why young cocoa that have not developed deep roots that can reach water table are always vulnerable to water stress in long dry seasons.



3.4. Stomatal conductance of cocoa clones

Figure 3 The stomatal conductance of the fully expanded leaf of cocoa clones during dry and wet seasons. Values represent the means of leaves measured repeated on two occasions. Bars shows the Least Significant Differences

The stomatal conductance of cocoa followed almost the same pattern with their stomatal transpiration. All clones produced their higher values during wet seasons. The individual cocoa clones performance and clones*seasons interactions were significant (P = 0.05). SCA 9 has the overall highest stomatal conductance value of 0.3241 mol m⁻²s⁻¹ during wet season and was significantly higher than the values of 0.3058 mol m⁻²s⁻¹ and 0.3052 mol m⁻²s⁻¹ produced by IMC 47 and N 38, respectively during the same period. Similarly, TC 2 had the overall highest stomatal conductance value of 0.3087 mol m⁻²s⁻¹ in dry season which was also significantly higher than 0.2778 mol m⁻²s⁻¹, 0.2767 mol m⁻²s⁻¹ and 0.2723 mol m⁻²s⁻¹ produced by SPEC 54,UF 67 and N 38, respectively during the same season (Fig. 3). There was an observation of relatively lower stomatal conductance in the dry seasons for all clones which revealed that less water was available to the plants during the period. Just like stomatal transpiration, it was also observed that although water availability was quite low during dry seasons, the stomata conductance was never completely stopped during the period of data collection.

3.5. The photosynthetic rate of cocoa clones

The effect of clone on the individual photosynthetic rates performance and clone*season interaction were also significant at (P = 0.05) respectively (Fig. 4). Apart from AMAZ 15, the individual photosynthetic rates of all cocoa clones were relatively higher during wet seasons than in dry seasons. For dry season performance, N 38 with the photosynthetic rate of 3.452 µmol m⁻²s⁻¹ was significantly lower than 3.872µmol m⁻²s⁻¹, 3.734 µmol m⁻²s⁻¹ and 3.787 µmol m⁻²s⁻¹ produced by SCA 9, F₃ Amazon and TC 2, respectively during the same period of time. Also, N 38 produced 4.141 µmol m⁻²s⁻¹ which was overall lowest photosynthetic rate in wet season and significantly lower than values produced by PA 150, SCA 9, F3Amazon and TC2 during the same season. Similar to stomatal transpiration and conductance performance, cocoa clones continued their active photosynthesis during the dry seasons, even though the activities were at much lower rates because of water stress. One way to explain this is that although the plants were relatively young, an adequate canopy had already been formed and the roots of cocoa were getting better established which made their actual water uptake to continue and perform photosynthetic activities.



Figure 4 The photosynthetic rates of the fully expanded leaf of cocoa clones during dry and wet seasons. Values represent the means of leaves measured repeated on two occasions. Bars shows the Least Significant Differences

3.6. Leaf relative water content of cocoa clones

The leaf relative water content (LWRC) of all cocoa clones is shown in Fig. 5.



Figure 5 Leaf Relative Water Content of the youngest fully expanded leaf of cocoa clones. Values represent the means of leaves measured repeatedly on two occasions. Bars show Standard Deviation

There were significant differences between clones in the 2020 dry season, 2021 wet season, 2021 dry season and 2022 wet season. The LRWC of AMAZ 15 (65.1 %) and F3 Amazon (64.9 %) were significantly lower than those of other cocoa clones in 2021 wet season while SPEC 54 and N 38 with LRWC 61.2 % and 60.7 % respectively were the significantly lowest values for 2021 dry season. Meanwhile, F3 Amazon, PA 150, SCA 6 and AMAZ 15 with 61.9 %, 64.2 %, 64.3 % and 65.1 % LRWC, respectively were significantly higher than those of other cocoa clones in 2022 wet season. Cocoa clones maintained a relatively stable leaf urgor and above-average LRWC throughout the period of data collection, irrespective of the seasonal availability of seasonal rainfall that helps maintain some photosynthesis and growth activities throughout the year during both seasons. Turgor pressure is controlled by osmotic adjustment [18, 19] and reflected in the variation of the individual LRWC performance of cocoa clones in this present study. This was in agreement with the reports of Martínez et al. [20] that for plant cells to exhibit normal activities and growth, its leaves must maintain high turgor even under low water availability. Balasimha [21] explained that LRWC is the performance of plant genetic make-up as influenced by the ambient environmental factors such as the temperature and relative humidity. Hence, the observation of significant clone*season interactions was responsible for lower leaf turgor of cocoa during dry seasons. Balasimha et al.[22] and Abo-Hammed [23]reported similar lower LRWC among cocoa clones during dry season conditions while LRWC of cocoa reported in the present study in Nigeria were lower than those recorded by Abo-Hammed [23] and Almeida et al. [24] in Brazil for the same seasons, values of cocoa LRWC reported in the present study in Nigeria were similar to those reported by Acheampong [25] in Ghana and could be explained as some exhibition of variation in the regional performance of cocoa LRWC.



3.7. Cuticular transpiration of cocoa clones

Figure 6 The cuticular transpiration of cocoa clones. Values represent the means of leaves measured repeatedly on two occasions. Bars show Standard Deviation

There were significant differences (P = 0.05) between clones in 2020 dry season, 2021 wet season and 2021 dry season but not in 2022 wet season. There was significant overall clone*season interaction but more variability between clones were recorded in the dry seasons than in the wet seasons. The rate of CT is directly depended on the deposit of

epicuticular wax on the leaf [26]. In cocoa, low CT normally result from the heavy epicuticular wax deposit that eventually help in reducing further water loss after the main transpiration has ceased or has extremely reduced. Low transpiration rates through the stomatal and cuticle are water saving mechanism in dry season [3] but lower stomatal transpiration reduces the photosynthetic rates and growth of cocoa. Clones like UF 676 and N 38 with relatively lower stomatal transpiration rates, lower stomatal conductance and low LRWC but high CT during dry seasons might not be able to maintain a good water balance during long dry seasons.

3.7. The stomatal density of cocoa clones



Figure 7 Samples of a cocoa leaf structure at CRIN as shown in the microscope when most of cocoa stomata are closed while (A) and when most of its stomata are opened (B)



3.8. The mean values of the stomatal density of sampled cocoa leaves

Figure 8 Means of stomatal density of the fully expanded leaf of cocoa clones during dry and wet seasons. Values represent the means of leaves measured repeated on two occasions. Bars shows the Least Significant Differences

There was a significant (P = 0.05) difference in the values of stomatal density between cocoa clones in the dry season as well as in their clone*season interactions but not in the wet season. Cocoa clones recorded relative higher stomata density values in the wet season than in dry season. There were variation in the performance of cocoa clones. For instance, TC 2 with 280 mm² in dry season was significantly higher than the 263 mm² recorded of MAN 15 at the same period. One explanation is that as trees continued to experience water stress, there is a time when an extensive decrease in their water content or an increase in water deficit reaches a threshold level which negatively affects their physiological processes and growth of cocoa [27]. The stomatal transpiration rates and stomatal conductance of cocoa clones increased with the values of their stomata density. This showed the direct influence of density of stomata pores

on the control of gas exchange performances of cocoa clones in agreement with the reports of Nunes [28]. Consequently, effective reduction in water loss is highly desirable for cocoa clones to survive water stress condition.

3.9. Relationship between stomatal transpiration and stomatal density of cocoa clones





The regression analysis showed that a linear, positive and significant relationship existed between the stomatal transpiration and stomatal density of all cocoa clones. This revealed that the observed higher stomatal transpiration recorded by cocoa in wet seasons responded to higher water status of the plants which resulted in higher CO_2 intake for higher photosynthetic rates during the period. With the established close link between its stomatal transpiration and stomata density, cocoa clone with a relatively lower stomatal density during dry season may likely be more tolerant to drought than those with higher stomatal density during the same period.



3.10. Water use efficiency of cocoa clones

Figure 10 Water Use Efficiency of the fully expanded leaf of cocoa clones. Values represent the means of leaves measured repeated on two occasions. Bars shows the Least Significant Differences

There was significant (P=0.05) clones and clone*season values among the water use efficiency (WUE) of the cocoa clones both in dry and wet seasons. WUE values were higher during dry season for the majority of cocoa clones, except for ICS 95, MAN 15 and N 38 which recorded their higher WUE during wet season. In dry season, AMAZ 15 recorded the

lowest WUE with 1.801 μ mol mmol⁻¹ which was significantly lower (P =0.05) than 2.093 μ mol mmol⁻¹, 2.1107 μ mol mmol⁻¹, 2.125 μ mol mmol⁻¹ and 2.126 μ mol mmol⁻¹ of PA 150, SCA 9, F3 Amazon and TC 2, respectively. In wet season, AMAZ 15 recorded the lowest WUE value of 1.693 μ mol mmol⁻¹ was significantly lower than 2.024 μ mol mmol⁻¹, 1.977 μ mol mmol⁻¹ and 1.98 μ mol mmol⁻¹ values recorded by SCA 9, F3 Amazon and TC 2, respectively. The present study revealed that the stomatal conductance, transpiration and photosynthetic rate of cocoa clones were reduced in the dry seasons but clones experienced increased WUE during the period. This confirmed a relative reduction in stomatal conductance and CO₂ assimilation which ensure a higher efficiency of available water by cocoa during water deficit. According to Dias *et al.* [29] plant physiological responses to soil water availability shows that clones that combine good growth ability with high WUE during dry season would be of great advantages for use in the drought-prone areas.

4. Conclusion

The significant effect of clone in the stomatal transpiration, stomatal conductance and photosynthetic rates of all cocoa clones is an indicative of genetic variability in their gas exchange characteristics. The observation of a significant effect of clone on WUE in this study may be a direct reflection of varied stomata densities and regulations among cocoa clones. Also, it was observed that transpiration cuticular transpiration did not have any significant influence on the WUE during dry season. Therefore, any cocoa clone that combines either high WUE and/or high LRWC with low cuticular transpiration during severe water stress condition may have better tolerance to drought.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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