

Physiological Effects of Drought Stress on Seedlings of Different Coffee Varieties

Shunjiao Xu¹, Liping Liu^{1,*}, Ruifang Wang¹, Yating Luo¹, Yufan Li

¹ Pu'er University, Pu'er, China

* Corresponding author

Abstract: To elucidate the impact of drought stress on the physiological characteristics of seedlings from different coffee varieties, seedlings of the small-bean varieties 'Typica', 'Geisha', 'Central America', and 'Ethiopia' were used as experimental materials. This study compared the physiological changes of seedlings from these four different coffee varieties under three levels of drought stress (75%–80%, 55%–60%, 35%–40%). Physiological indicators such as chlorophyll content, soluble sugar content, proline content, peroxidase (POD) activity, and malondialdehyde (MDA) content were measured. The results indicated that with the increase in the degree of water stress, the contents of soluble sugars, proline, chlorophyll, POD activity, and MDA content all showed varying degrees of initial decline followed by an increase; suggesting that the intensification of drought stress has a certain impact on plant drought resistance. Under moderate stress (55%–60%) compared with mild (75%–80%) and severe (35%–40%) stress, the overall growth rate of indicators among varieties was higher, indicating better plant stress resistance. Based on comprehensive analysis of drought resistance indicators, principal component analysis, and subordinate function comprehensive evaluation, the drought resistance capability of the varieties was ranked from highest to lowest as follows: Typica coffee > Geisha coffee > Ethiopian coffee > Central American coffee.

Keywords: Varieties; Coffee Seedlings; Drought Stress; Physiology.

1. Introduction

Coffee (*Coffea arabica* L.) is a small shrub of the Rubiaceae family and *Coffea* genus, which prefers cool, moist environments and is sensitive to drought [1-2]. Early research has demonstrated that drought treatment has a strong effect on the seedling stage of small-grain coffee varieties, with a significant connection between the seedlings' drought resistance and their osmotic adjustment capacity and antioxidant enzyme activity [3]. Mild water stress can cause a decrease in the photosynthetic rate of small-grain coffee leaves and a reduction in coffee yield [4-6]. Studies have also shown that initially, drought-affected soil has a significant impact on the lower lateral branches' leaves of the plant, but the upper branches' leaves maintain a better water supply [7]. Current research on the drought resistance of various small-grain coffee varieties planted in Yunnan is not comprehensive. This study evaluates the drought resistance of different small-grain coffee varieties by comparing their physiological changes under drought stress, providing a basis for coffee cultivation and promotion under local drought conditions.

2. Materials and Methods

2.1. Experimental Materials

Four varieties of coffee seedlings from a fine seedling breeding base of small-grain coffee in Yunnan Province were selected as experimental materials: 'Typica', 'Geisha', 'Central America', and 'Ethiopia'.

2.2. Experimental Methods

In June 2023, coffee seedlings of consistent growth, eight per variety, totaling 32 plants, were acclimatized for one week and then transplanted into pots in the greenhouse of Pu'er College. After 15 days of cultivation, they were subjected to drought treatment. The potting medium consisted of a

seedling substrate: sand: loam = 3:3:4. According to Hsiao's method [8], three levels of water stress were applied to the coffee seedlings: mild drought (soil moisture content of 75%–80%), moderate drought (soil moisture content of 55%–60%), and severe drought (soil moisture content of 35%–40%).

2.3. Physiological Index Measurement and Analysis

On the seventh day of the drought treatment, the contents of malondialdehyde (MDA), proline, soluble sugar, peroxidase (POD), and chlorophyll were measured using colorimetry. Data analysis of physiological indices was conducted using Microsoft Excel software, and statistical analysis was performed using SPSS 21 software. The analysis of coffee seedlings' drought tolerance characteristics referred to the subordinate function [9] and principal component analysis methods proposed by Bai Zhiying et al.

3. Results and Analysis

3.1. The Impact of Drought Stress on Osmotic Adjustment Substances in Different Coffee Varieties

The results of physiological change indicators are shown in Table 1. Under mild drought conditions, the soluble sugar content in 'Ethiopia' coffee increased by 1.194%, 0.956%, and 1.152% compared to 'Typica', 'Geisha', and 'Central America' coffee, respectively. Under moderate drought conditions, 'Ethiopia' coffee showed the highest increase in soluble sugar content compared to 'Typica', 'Geisha', and 'Central America', with increases of 2.157%, 1.64%, and 1.38%, respectively. Under severe drought conditions, there were no significant differences between 'Geisha' and 'Central America'; 'Typica' showed a slow increase when compared to 'Geisha', 'Central America', and 'Ethiopia'. This indicates that the severity of

drought stress affects the plant's internal soluble sugar content, which is a manifestation of plant stress resistance [10]. The drought resistance capability based on changes in soluble sugar content of coffee seedlings can be ranked as follows: Central America coffee > Geisha coffee > Ethiopia coffee > Typica coffee.

Proline is one of the most important osmotic adjustment substances within plants. When plants are subjected to severe drought, the internal content of proline can change significantly [10]. 'Typica' and 'Central America' showed significant changes under mild, moderate, and severe drought conditions, with 'Typica' displaying a rise-fall trend with significant amplitude, while 'Central America' showed a rising trend with a larger increase. 'Geisha' and 'Ethiopia' showed no significant changes within the varieties. Under mild drought conditions, 'Typica' increased by 4.13 times, 8.61 times, and 2.06 times compared to 'Geisha', 'Central America', and 'Ethiopia', respectively. Under moderate and severe drought conditions, 'Central America' showed larger increases compared to 'Typica', 'Geisha', and 'Ethiopia', with increases of 8.51 times, 4.46 times, 2.05 times and 10.74 times, 4.78 times, 2.52 times, respectively. Under moderate drought conditions, the inter-varietal accumulation of proline was higher than under mild and severe drought conditions, indicating that drought stress affects the internal content of proline. From the changes in proline content, the drought resistance can be ranked as follows: Central America coffee > Ethiopia coffee > Geisha coffee > Typica coffee.

3.2. The Impact of Drought Stress on MDA Content in Different Coffee Varieties

Malondialdehyde (MDA) is an important component of peroxidation substances, related to the effects of stress environments on plants and the production of reactive oxygen species similar to the peroxidation effect within the plasma membrane. The changes among different coffee varieties were significant, with varieties having higher resistance capability showing smaller increases and those with lower resistance capability showing larger increases [11]. Under mild drought conditions, 'Typica' showed a larger increase in MDA content compared to 'Geisha', 'Central America', and

'Ethiopia', with increases of 312.8%, 461.4%, and 106%, respectively. Under moderate and severe drought conditions, 'Typica', 'Geisha', and 'Central America' all showed an increasing trend. Under moderate drought conditions, the MDA content was lower than under mild and severe conditions, indicating lesser damage to the plasma membrane and stronger resistance. The MDA content under drought stress can reflect the extent of adversity damage to the plant. Based on the increase in MDA content, the drought resistance can be ranked as follows: Ethiopia coffee > Central America coffee > Geisha coffee > Typica coffee.

3.3. The Effect of Drought Stress on Antioxidant Enzyme Activity in Different Coffee Varieties

The correlation between POD levels and seedling drought tolerance has always been a subject of debate [12]. It is generally believed that varieties with lower drought tolerance exhibit significant increases in POD activity [13]. Antioxidant enzymes can eliminate H₂O₂ and induce the peroxidation of unsaturated fatty acids. As the intensity of drought stress gradually exceeds the defense capability of coffee seedlings, the metabolic balance of reactive oxygen species is impaired, leading to excessive accumulation of reactive oxygen species that affect and damage the enzyme structure. Both 'Central America' and 'Ethiopia' coffee varieties showed an initial increase followed by a decrease, with 'Central America' increasing by 38.9% and 58.6% under moderate drought conditions compared to mild and severe drought treatments. Under moderate and severe drought stress, 'Typica' and 'Geisha' showed clear changes compared to 'Central America' and 'Ethiopia', with 'Typica' and 'Geisha' increasing at a slower rate, while 'Central America' and 'Ethiopia' showed a significant decrease. The drought resistance based on changes in POD activity of coffee seedlings can be ranked as: Geisha coffee > Typica coffee > Ethiopia coffee > Central America coffee.

3.4. The Effect of Drought Stress on Photosynthetic Characteristics of Different Coffee Varieties

Table 1. Changes in Physiological Indicators of Coffee Seedlings Under Different Drought Stresses

Variety	Drought Stress Level	Soluble Sugar Content (%)	Proline Content (ug/g FW)	MDA Content (umol/g)	POD Activity (U/g)	Chlorophyll Content (mg/g FW)
Typica (Geisha)	Mild Drought	3.223	192.667	0.010	1.879	1.228
	Moderate Drought	4.236	174.333	0.003	0.854	1.904
	Severe Drought	4.425	185.667	0.007	0.919	2.070
Geisha	Mild Drought	2.985	795.333	0.020	3.024	0.622
	Moderate Drought	3.719	91.333	0.005	1.827	0.699
	Severe Drought	2.258	82.667	0.007	2.021	0.766
Central America	Mild Drought	3.027	92.333	0.009	2.059	0.660
	Moderate Drought	3.855	777.333	0.003	2.861	0.420
	Severe Drought	4.496	888.000	0.004	1.804	0.625
Ethiopia	Mild Drought	4.179	386.000	0.008	2.558	0.706
	Moderate Drought	5.876	379.333	0.006	2.291	0.734
	Severe Drought	3.507	352.000	0.004	1.270	0.807

Under mild drought conditions, the chlorophyll content in 'Geisha' compared to 'Typica', 'Central America', and

'Ethiopia' increased by 97.4%, 86%, and 73.9%, respectively. Under moderate drought conditions, the increases were

172.4%, 353.3%, and 159.4%, respectively. Under severe drought conditions, the increases were 170.2%, 231.2%, and 156.5%. This indicates that the maximum accumulation of chlorophyll within the plant body can enhance coffee resistance better. Based on the changes in chlorophyll content of coffee seedlings of different varieties, the drought resistance strength can be ranked as: Geisha > Ethiopia coffee > Typica coffee > Central America coffee.

3.5. Correlation Analysis between Measured Indicators and Principal Components

The correlation analysis between indicators is presented in Table 2, and the results of the principal component analysis are shown in Table 3.

Table 2. Correlation Analysis of Physiological Indicators of Coffee Seedlings of Different Varieties Under Drought Stress

		Soluble Sugar Content	Proline Content	MDA Content	POD Activity
Proline Content	Pearson Correlation	0.345			
	Significance (One-sided)	0.136			
MDA Content	Pearson Correlation	-0.366	0.177		
	Significance (One-sided)	0.121	0.291		
POD Activity	Pearson Correlation	-0.206	0.525*	0.501*	
	Significance (One-sided)	0.260	0.040	0.049	
Chlorophyll Content	Pearson Correlation	0.305	-0.449	-0.141	-0.787**
	Significance (One-sided)	0.167	0.071	0.331	0.001

Note: * indicates significance at the 0.05 level; ** indicates significance at the 0.01 level. A negative R value indicates a negative correlation, and vice versa. R represents correlation; sig indicates significance.

Table 3. Results of the Principal Component Analysis

Principal components	Eigenvalue	Contribution ratio (%)	Cumulative contribution ratio (%)
1	2.419	48.372	48.372
2	1.409	28.184	76.557

The first principal component has an eigenvalue of 2.419, accounting for 48.372% of the variance, with corresponding eigenvectors including MDA content, POD activity, proline content, chlorophyll content, and soluble sugars. Except for POD activity and chlorophyll content, which are negatively correlated, all other indicators are positively correlated, indicating that MDA content, proline content, and soluble sugars correspond to better growth and stronger resistance in different coffee seedling varieties, thus named the osmotic factor. The second principal component has an eigenvalue of 1.409, accounting for 28.184% of the variance, with

corresponding eigenvectors including proline content, chlorophyll content, and soluble sugar content, with proline content negatively correlated, while chlorophyll and soluble sugar content are positively correlated, named photosynthesis-related indicators. Through principal component analysis of the indicators of different coffee seedling varieties, we can use the osmotic factor and photosynthesis-related indicators to evaluate differences in drought resistance performance among different coffee seedling varieties.

Table 4. Factor Loading Matrix

Drought Resistance Indicators	Component 1	Component 2
MDA Content	0.946	0.050
POD Activity	-0.845	-0.056
Proline Content	0.577	-0.407
Chlorophyll Content	-0.319	0.876
Soluble Sugar Content	0.612	0.686

3.6. Drought Tolerance Evaluation of Different Coffee Varieties Based on Subordinate Function

The drought tolerance subordinate function method was used, taking the average of the subordinate degrees of all main indicators as the comprehensive evaluation criterion for the drought tolerance potential of coffee. The subordinate function values were calculated using the average values of each indicator under drought conditions, and then the average of these subordinate function values was used for a comprehensive comparison of drought tolerance capacity

among different coffee varieties, ultimately determining the degree of drought tolerance. The subordinate function formula is as follows:

$$U(X_i) = \frac{X_i - X_{\min}}{X_{\max} - X_{\min}} \quad (1)$$

Where $U(X_i)$ is the subordinate function value, X_i is the value of a particular indicator, and X_{\max} and X_{\min} are the maximum and minimum values of that indicator, respectively. If an indicator is negatively correlated with the overall

assessment result, a reverse subordinate function is used for quantitative transformation, with the calculation formula remaining the same.

According to Table 5, the drought tolerance subordinate function values for 'Geisha', 'Typica', 'Central America', and

'Ethiopia' coffee are 0.995 (U), 2.657 (U), 0.773 (U), and 0.882 (U), respectively. The ranking of drought tolerance capacity from highest to lowest is as follows: Typica coffee > Geisha coffee > Ethiopia coffee > Central America coffee.

Table 5. Comprehensive Evaluation of Drought Tolerance Subordinate Degree (U) of Coffee Seedlings of Different Varieties

Indicator	Variety			
	U (Geisha)	U (Typica)	U (Central America)	U (Ethiopia)
MDA Content	0.145	0.596	0.695	1.043
POD Activity	0.100	1.067	0.136	0.526
Proline Content	0.400	10.477	0.344	0.424
Chlorophyll Content	2.186	0.654	2.153	2.285
Soluble Sugar Content	2.142	0.493	0.538	0.130
Overall Evaluation	0.995	2.657	0.773	0.882
Rank	2	1	4	3

(Note: There seems to be a slight inconsistency in the narrative regarding the ranking of coffee varieties based on their drought tolerance capacities as previously mentioned and the values given in the section above. Please ensure that the data and names correspond correctly to the intended information.)

4. Conclusion and Discussion

Drought stress affects the proline content within plants, and an increase in proline content is an indicator of plant stress resistance. The variation in soluble sugar content in response to drought stress demonstrates the differences in osmotic adjustment among different varieties; plants accumulate a large amount of osmotic adjustment substances under drought stress, which enhances their drought resistance. The membrane permeability reflected by MDA content indicates the plant's ability to cope with drought stress, related to membrane permeability. Higher MDA content indicates stronger membrane permeability and greater plant damage. Antioxidant enzymes can eliminate H₂O₂, thereby mitigating the damage from external stress environments. Changes in photosynthetic characteristics under drought stress reflect the growth condition of plants; higher chlorophyll content indicates stronger photosynthetic capacity and resistance.

Drought tolerance identification is the process of screening, assessing, and classifying plant types of coffee based on their drought tolerance potential. This process provides good germplasm data for breeding [14-15]. The comprehensive index value of a variety does not solely determine its drought resistance capability. By averaging the contribution rates of two comprehensive indicators, the corresponding subordinate function value is derived, and comparing these comprehensive indices provides an overall score for the drought resistance potential of each variety. This makes the greatest difference in drought resistance potential among varieties comparable [16], establishing a basis for ranking plant drought resistance traits, in order: Geisha coffee > Typica coffee > Ethiopia coffee > Central America coffee.

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