AATCC Review

24 March 2025: Received
14 May 2025: Revised
21 May 2025: Accepted
24 June 2025: Available Online

https://aatcc.peerjournals.net/

Original Research Article   Open Access

Assessment of Root Trait Variability Among Graft Combinations in Coffea arabica Under Water Stress

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ABSTRACT

Coffee (Coffea arabica) is a major global commodity, yet its productivity is increasingly challenged by water scarcity, especially in rainfed cultivation systems. This study investigated the physiological and morphological responses of 16 grafted coffee combinations and their respective parental lines under 70% water deficit conditions using a rain-out shelter over three consecutive years (2015–2018). Significant genetic variability was observed in root traits, total dry matter (TDM) and water use efficiency (WUE). Among the grafts, S.4538/Sln.6 recorded the highest TDM (112.8 g plant^{-1}), root biomass (27.0 g plant^{-1}) and shoot biomass (44.9 g plant^{-1}). The highest root-to-shoot ratio was found in Sln.6/Sln.10 (1.05), while S.4538/Sln.6 showed the lowest (0.60), indicating diverse carbon allocation patterns under stress.Water use efficiency varied significantly among combinations, with Sln.11/Sln.11 achieving the maximum WUE (5.84 g kg^{-1}), followed closely by Sln.11/Sln.6 (5.82 g kg^{-1}), Sln.10/Sln.6 (5.73 g kg^{-1}) andTaffarikela/Sln.6 (5.61 g kg^{-1}). The highest Net Assimilation Rate (NAR) and Mean Transpiration Rate (MTR) were recorded in Taffarikela/C x R (4.39 g dm^{-2} day^{-1} and 23.27 mg dm^{-2}, respectively). Z-score-based classification further revealed that grafts like S.4538/Sln.6, Sln.6/Taffarikela and Sln.10/Sln.6 consistently combined high WUE with strong root development. A negative correlation between MTR and WUE (r = –0.68) confirmed that efficient stomatal regulation contributed to water-saving strategies under drought.These findings underscore the potential of grafting drought-sensitive but agronomically elite arabica lines onto physiologically resilient rootstocks. Such combinations offer a dual advantage of improved water acquisition and utilization, providing a viable strategy for enhancing coffee resilience and yield stability under increasing climatic variability.

Keywords: *Coffea arabica, Grafting, Drought tolerance, Water use efficiency (WUE), Root-to-shoot ratio, Root traits, carbon allocation, physiological responses.*

1. INTRODUCTION

Coffee is one of the most important global commodities, playing a vital role in international trade. Commercial coffee production primarily relies on two species: *Coffea arabica* (arabica) and *Coffea canephora* (robusta). Among these, arabica is particularly prized for its superior cup quality. However, arabica is genetically narrow, self-pollinating and highly susceptible to several destructive diseases, including leaf rust, berry blotch and root-related pathogens [1]. Despite having a deeper root system than robusta, which gives it some resilience to drought, arabica displays significant variability in water stress tolerance across cultivars and regions [2].

Even in high rainfall zones of India, where annual precipitation exceeds 2000 mm, coffee crops often experience seasonal droughts lasting up to six months [3]. During extended dry spells, floral abscission is occasionally observed, which can substantially reduce yields. Although irrigation mitigates drought-induced stress, many Indian coffee plantations lack adequate irrigation infrastructure.

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DOI: https://doi.org/10.21276/AATCCReview.2025.13.03.272

Moreover, arabica shows a limited positive response to irrigation compared to robusta. Inappropriate irrigation management can exacerbate water stress rather than alleviate it, ultimately reducing plant vigor and productivity [4].

Water limitation is known to negatively influence growth and productivity by altering plant physiology, particularly the root-to-shoot ratio and carbon assimilation pathways [5; 6]. Under stress conditions, plants often reduce water and nutrient uptake from the roots, leading to reduced stomatal conductance and diminished photosynthetic capacity, which in turn impacts carbohydrate allocation to reproductive structures, including fruits [7].

The strategy of *regulated deficit irrigation* (RDI), first conceptualized by Chalmers and colleagues, has shown promise in enhancing water use efficiency and optimizing yield quality by applying moderate water stress at specific phenological stages [8]. In crops like watermelon and eggplant, RDI has improved reproductive allocation while restraining excessive vegetative growth [9].

Another promising approach involves the use of grafting to improve drought tolerance and water use efficiency. Grafting has traditionally been used to control soilborne diseases in vegetables, but recent developments have expanded its application to abiotic stress tolerance, nutrient uptake and heavy metal exclusion [10;11]. For instance, grafting has been employed to increase resilience against salinity, flooding, boron toxicity and micronutrient deficiencies [12; 13].

Despite these advances, limited research exists on how grafting affects the physiological and agronomic responses of grafted mini-watermelons under water-deficient conditions. The current study seeks to explore this gap by assessing the impact of water stress on grafted coffee seedlings. Six-month-old plantscomprising 16 graft combinations and their respective parental lineswere cultivated under controlled environmental conditions with a 70% water deficit using a rain-out shelter. The research focused on evaluating water relations, root morphological traits and overall growth parameters between the grafted plants and their parents.

2. Materials and Methods

2.1 Experimental Site and Climate Conditions

A three-year field experiment was conducted during the 2015–2018 period at the Central Coffee Research Institute, located in Chikkamagaluru District, Karnataka, India (13°22′2″ N, 75°25′14″ E, altitude 890 m). This region experiences a humid subtropical climate, with an average annual rainfall of approximately 1548 mm, a mean maximum temperature of 27.9 °C and a mean minimum of 17.3 °C. Relative humidity averaged around 85.7% and daily sunshine duration ranged between 4.5 and 8.7 hours, representing typical regional conditions for coffee cultivation [14].

2.2 Plant Material and Nursery Management

Grafted coffee seedlings, comprising 16 rootstock-scion combinations and their respective parental lines, were raised in polybags (35 cm × 25 cm) filled with a 6:3:1 mixture of farmyard manure, sand and forest (jungle) soil. Plants were maintained under 40% filtered shade with routine nutrient and plant protection management as per standard nursery protocols [15]. Grafting was performed using the wedge-cleft method at the button stage. Details of the grafts used are given in Table 1.

After six months, healthy and uniform grafted seedlings were transplanted into large battery containers for further evaluation under controlled drought conditions. A rain-out shelter (ROS) was employed to simulate a 70% water stress regime, maintained consistently throughout the experiment to mimic severe drought conditions.

2.3 Experimental Design and Plant Growth Monitoring

To avoid limitations in root expansion and mimic natural growth behavior, plants were grown in adequately sized containers. Research has shown that approximately 90% of the root mass of coffee develops within the topsoil under field conditions [16]. Five plants per graft combination were randomly selected for biometric measurements. For destructive sampling, three plants per treatment were harvested at different growth stages and partitioned into roots, stems and leaves. Morphological parameters such as initial root length, shoot length and leaf area were recorded. Following air-drying at 80 °C until a constant weight was reached, the dry biomass of each plant part was measured and expressed in grams per plant.

2.4 Water Stress Regime and Transpiration Measurement

Soil moisture levels were managed gravimetrically to maintain 70% field capacity (FC). The water required to maintain this regime (RQ70) was calculated using the formula:

RQ 70= WFC 70 –X, Where: WFC_{70} is the weight of the soil-filled container at 70% FC. X is the current container weight at time *t*.

Daily water loss due to transpiration was estimated by weighing the containers using a precision mobile weighing system.

RQ 70= WFC 70 –X,

Water Use Efficiency and Growth Analysis

RQ 70= WFC 70 –X,

2.5 Statistical Analysis

The experimental data were subjected to analysis of variance (ANOVA) following the procedure outlined by Panse and Sukhatme (revised by [17]). Differences among treatment means were evaluated at the 5% significance level ($P < 0.05$), while a 1% level ($P < 0.01$) was used for more sensitive assessments. When the F-test was significant, the critical difference (CD) was calculated to compare treatment means.

Correlation coefficients among growth traits, water use parameters (WUE, CWT), biomass accumulation and yield components were determined using Pearson's method. Significance levels for correlation were assessed for n-1 degrees of freedom at $P < 0.05$ and $P < 0.01$ levels, as per modern adaptations of Snedecor and Cochran's methods [18].

3. Results and Discussion

3.1 Root Biomass and Morphological Traits Under Water Stress

Root development and stomatal regulation are fundamental to biomass accumulation and plant resilience under drought conditions. This study highlighted significant genetic variability among coffee graft combinations with respect to root dry weight, shoot biomass and root-to-shoot (R:S) ratios under 70% water stress conditions (Table 2). Among the combinations evaluated, the graft S.4538/Sln.6 produced the highest root (27.0 g plant^{-1}) and shoot biomass (44.9 g plant^{-1}), followed closely by Sln.6/Taffarikela, Sln.10/Sln.6 andSln.6/Sln.10. In contrast, the lowest biomass was recorded in Taffarikela/Sln.10 (14.3 g plant^{-1}) and Taffarikela/S.4202 (19.0 g plant^{-1}).

Total biomass production was significantly greater in grafts with higher root biomass, indicating the crucial role of root architecture in drought adaptation. The superior performance of S.4538/Sln.6 (112.8 g plant^{-1}) demonstrates how enhanced root systems contribute to greater water uptake and dry matter accumulation, especially under limited moisture availability. This is consistent with findings in other crops where larger root systems facilitate deep water extraction, improving plant water potential and supporting biomass maintenance under drought [19; 20].

The highest R:S ratio was observed in Sln.6/Sln.10 (1.05), followed by Sln.10/Sln.6 (0.89), while S.4538/Sln.6 had the lowest (0.60). Such modifications in biomass partitioning under water deficit are well-documented adaptive responses that enable better water foraging capacity [21; 22]. Grafts were classified into four groups based on root biomass, with those having higher root weights under control conditions also maintaining superior root traits under stress (Table 3). This pattern was evident in a 3 to 4fold difference in average root biomass across the groups (Fig. 1 and Fig. 2). The consistency of these root traits under stress reflects their genetic stability and relevance for selection in breeding programs.

3.2 Water Use Efficiency (WUE) and Growth Metrics

Improving root traits alone may be insufficient for drought resilience in rainfed crops like coffee. Excessive root growth can accelerate soil moisture depletion, potentially aggravating stress [23]. Therefore, it is essential to assess water use efficiency (WUE)—the ratio of total dry matter (TDM) produced to cumulative water transpired (CWT).

Among all combinations, Sln.11/Sln.11 recorded the highest WUE (5.84 g kg^{-1}), followed by Sln.11/Sln.6 (5.82 g kg^{-1}), Sln.10/Sln.6 (5.73 g kg^{-1}) andTaffarikela/Sln.6 (5.61 g kg^{-1}) (Table 4). These results align with earlier studies reporting that genotypes with enhanced WUE perform better under drought due to efficient stomatal regulation and photosynthetic carbon fixation [24; 25].

Additionally, the Net Assimilation Rate (NAR) and Mean Transpiration Rate (MTR) were used to understand physiological responses. The highest NAR and MTR were found in Taffarikela/C x R (4.39 g dm^{-2} day^{-1} and 23.27 mg dm^{-2}, respectively), followed by Sln.6/Sln.10. These values emphasize the role of genotypic differences in maintaining carbon gain under moisture stress. Lower transpiration rates in some high-WUE grafts indicated improved stomatal control, supporting earlier findings that stomatal behavior significantly influences WUE over photosynthetic capacity alone [26].

3.3 Classification Based on WUE and Root Traits

Grafts were classified into high, medium and low WUE categories. High WUE types (Sln.11/Sln.6, Sln.10/Sln.6, Taffarikela/Sln.6) averaged 5.49 g kg^{-1}, while medium and low types recorded 4.90 g kg^{-1} and 3.97 g kg^{-1}, respectively (Table 5). A standardized Z-score transformation based on TDM, CWT, R:S ratio and WUE helped categorize grafts into four classes (Table 6; Fig. 3a and Fig. 3b). Grafts such as S.4538/Sln.6 and Sln.6/Taffarikela not only maintained high root biomass but also exhibited high WUE, providing dual advantages of deep water access and efficient water utilization.

Interestingly, some grafts such as Sln.6/Sln.10 also showed high WUE despite moderate or high MTR, suggesting that efficiency in carbon gain per unit of water was not strictly dependent on transpiration reduction. These outliers are crucial for breeding as they represent combinations with high physiological efficiency [27].

A strong inverse relationship between MTR and WUE (Table 7) reinforces the idea that genotypes with controlled water loss exhibit better drought adaptation. Since both CWT and WUE contribute directly to biomass, grafts with high values in both traits achieved superior TDM.

4. Conclusion

This study provides robust evidence that grafting offers a promising avenue for improving drought resilience and water use efficiency (WUE) in *Coffea arabica*. Significant genotypic variation was observed among the 16 graft combinations in terms of root architecture, total dry matter accumulation and physiological traits under 70% water stress. Graft combinations such as S.4538/Sln.6, Sln.10/Sln.6 and Sln.6/Taffarikela consistently demonstrated superior performance by maintaining high root biomass, elevated root-to-shoot ratios and enhanced WUE. These traits collectively contributed to higher biomass productivity under stress conditions.

The study also highlights that optimizing root traits alone is not sufficient; rather, the integration of root efficiency with stomatal regulation and photosynthetic capacity is critical for sustained performance in water-limited environments. The identification of grafts with both high root biomass and high WUEwithout excessive transpirationunderscores their potential for future breeding and rootstock selection programs.

Given the increasing vulnerability of rainfed coffee systems to erratic rainfall and prolonged droughts, deploying such physiologically efficient graft combinations can play a pivotal role in climate-resilient coffee production. These findings not only enhance our understanding of genotype × environment interactions under moisture stress but also offer practical insights for sustaining coffee yields in a changing climate.

5. Article Type

This manuscript is a Research Article based on a field study evaluating the new elite arabica coffee hydrides under different moisture regimes.

6. Author Contribution

All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by Somashekhargouda Patil, Yashaswini K P, Yashasvi H R and M. Senthilkumar. The first draft of the manuscript was written by Somashekhargouda Patil and all authors commented on previous versions of the manuscript. All authors read and approved the final version of the” manuscript.

7. Conflict of Interest

The authors declare that they have no conflicts of interest.

8. Acknowledgment

The authors are grateful to the scientists of the Division of Plant Breeding and Genetics for identifying and providing the elite hybrid plants for evaluating their physiological efficiency and to the parent organization, Central Coffee Research Institute, Chikmagaluru, for providing all facilities to carry out the research study.

Table 1 - Graft Combinations, Rootstock and Scion Identity and Parental Traits for Root Biomass and Water Use Efficiency in Coffee

Grafts	Rootstock	Scion	Parents used and their known traits
1	Sln.6	Sln.11	Sln.10: Low root and Low WUE Sln.6: High root and High WUE Taffarikela: High root and High WUE S.4202: Low root and low WUE Sln.11: Low root and high WUE WUE-Water Use Efficiency
2	Sln.11	Sln.6	
3	Sln.10	Sln.6	
4	Sln.6	Sln.10	
5	Taffarikela	Taffarikela	
6	Taffarikela	S.4202	
7	Taffarikela	Sln.10	
8	Sln.6	Sln.6	
9	Taffarikela	Sln.6	
10	Sln.11	Sln.11	
11	Sln.6	Taffarikela	
12	CxR	CxR	
13	Taffarikela	CxR	
14	S.4538	Sln.6 (SWG)	
15	Sln.10	Sln.6 (SWG)	
16	Sln.6	Sln.10	

Table 2-Genetic variability different RS/Scion combinations to growth parameters

Sl No	Root Stock/Scion	Root weight (gms)	Shoot weight (gms)	Root: Shoot ratio	Total dry matter (gms)
01	Sln.6/Sln.11	22.6	29.8	0.758	73.2
02	Sln.11/Sln.6	17.9	23.9	0.748	57.8
03	Sln.10	14.0	14.7	0.952	41.3
04	Sln.10/Sln.6	25.2	34.1	0.739	82.3
05	Sln.6/Sln.10	24.5	23.2	1.056	70.2
06	Taffarikela/Taffarikela	14.4	17.9	0.804	42.7
07	Taffarikela	26.9	38.9	0.691	92.5
08	Sln.6	20.4	30.6	0.666	72.1
09	Taffarikela/S.4202	17.0	19.0	0.894	58.8
10	Taffarikela/Sln.10	14.3	21.2	0.674	54.4
11	Sln.6/Sln.6	20.3	31.0	0.654	74.2
12	Taffarikela/Sln.6	22.0	27.5	0.800	74.9
13	S.4202	24.7	23.3	1.064	76.8
14	Sln.11/Sln.11	17.1	22.3	0.771	50.6
15	Sln.11	9.2	13.5	0.681	35.7
16	Sln.6/Taf	25.3	31.8	0.795	84.1
17	C × R/C × R	29.1	31.8	0.915	68.5
18	Taffarikela/C × R	16.6	23.5	0.706	77.4
19	S.4538/Sln.6	27.0	44.9	0.601	112.8
20	Sln.10/Sln.6 (SWG)	23.2	25.9	0.895	83.3
21	Sln.6 (SWG)/ Sln.10	20.1	33.3	0.603	81.1
	CD@5%	11.4	NS	NS	31.6

Table 3- Classification of grafts based on absolute root weight (gms) under stress

Group	Root wt in gms/plant	Graft combinations
Gr.I	>20	Sln.6/Sln.11, Sln.10/Sln.6, Sln.6/Sln.10,Taffarikela, Sln.6, Sln.6/Sln.6, Taffarikela/Sln.6, S.4202, Sln.6/Taffarikela, C×R/C×R, S.4538/Sln.6, Sln.10/ Sln.6 (SWG), Sln.6(SWG)/Sln.10
Gr II	15-20	Sln.11/Sln.6, Taffarikela/S.4202, Sln.11/Sln.11, Taffarikela/C×R
Gr III	10-15	Sln.10, Taffarikela/Sln.10, Taffarikela/Taffarikela
Gr IV	5-10	Sln.11

Table 4- Genetic variability in WUE associated traits of different RS/Scion combinations

Sl No	Root Stock/Scion	Water Use Efficiency (WUE) (g/kg^{-1})	Leaf Area Duration (dm^2)	Mean transpiration rate (ml/dm^2)	Net Assimilation rate (mg/dm^2)
01	Sln.6/Sln.11	4.44	4673.9	3.41	15.21
02	Sln.11/Sln.6	5.84	3800.3	2.62	14.27
03	Sln.10	4.38	3741.4	2.57	10.6
04	Sln.10/Sln.6	5.73	5164.3	2.81	15.18
05	Sln.6/Sln.10	4.56	5042.6	2.98	13.41
06	Taffarikela/Taffarikela	5.3	3169.9	2.43	12.63
07	Taffarikela	5.16	5866.8	3.04	15.48
08	Sln.6	4.47	4948.3	3.13	13.9
09	Taffarikela/S.4202	4.23	4971.2	2.74	11.17
10	Taffarikela/Sln.10	5.1	3949.5	2.9	11.76
11	Sln.6/Sln.6	4.74	4519.2	3.28	15.57
12	Taffarikela/Sln.6	5.61	4822.2	2.8	14.63
13	S.4202	4.85	5432.5	2.95	14.24
14	Sln.11/Sln.11	6.54	3476.1	2.5	13.45
15	Sln.11	7.2	2559.1	2.08	13.06
16	Sln.6/Taf	5.19	5898.2	3.04	15.58
17	C × R/C × R	4.67	5522.5	3.16	12.41
18	Taffarikela/C × R	4.07	4710.7	4.39	23.27
19	S.4538/Sln.6	4.44	6837.8	3.69	16.36
20	Sln.10/Sln.6 (SWG)	3.97	5509.4	3.82	14.86
21	Sln.6 (SWG)/ Sln.10	4.3	4547.7	4.13	17.75
	CD@5%	1.12	1766	NS	5.44

Table 5- Classification of grafts based on Water Use Efficiency (WUE) under stress

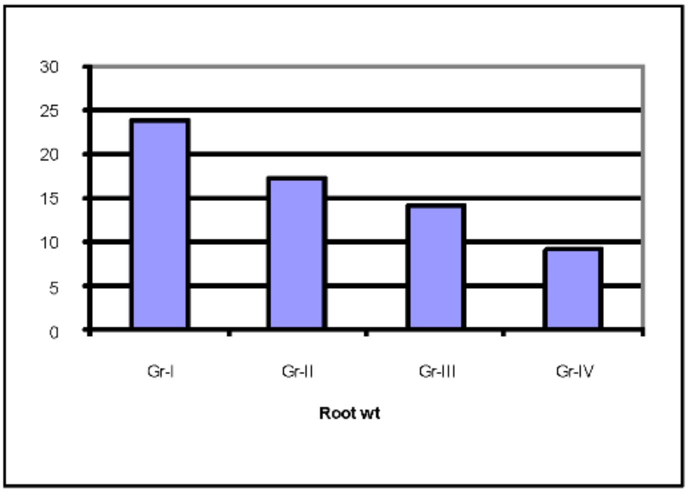
Group	Graft combinations
High (5-6)	Sln.11/Sln.6, Sln.10/Sln.6, Taffarikela /Taffarikela, Taffarikela, Taffarikela /Sln.10, Taffarikela /Sln.6, Sln.11/Sln.11, Sln.11, Sln.6/Taffarikela
Medium (4-5)	Sln.6/Sln.11, Sln.10, Sln.6/Sln.10, Sln.6, Taffarikela/S.4202, Sln.6/Sln.6, S.4202, C × R/C × R, Taffarikela/ C × R, S.4538/Sln.6 (SWG), Sln.6(SWG)/Sln.10
Low (<4)	Sln.10/Sln.6 (SWG)

Table 6- Classification of grafts based on Water Use Efficiency (WUE) and root wt under stress

High root biomass & High WUE	Sln.10/Sln.6, Taffarikela, Taffarikela /Sln.6, Sln.6/Taffarikela, Sln.11/Sln.11
High root biomass & low WUE	Sln.10/Sln.6 (SWG)
Low root biomass & high WUE	Taffarikela /Sln.10, Taffarikela /Taffarikela, Sln.11
Low root & med/low WUE	Sln.10

Table 7-Correlation coefficients of the relationship of WUE with a few important root characteristics and physiological parameters among the grafts

Index	TDM	CWT	WUE	RDW	MTR	NAR
TDM	1.000					
CWT	0.864**	1.000				
WUE	-0.297^{NS}	-0.652**	1.000			
RDW	0.736**	0.587**	-0.054^{NS}	1.000		
MTR	0.715**	0.928**	-0.685**	0.479**	1.000	
NAR	0.814**	0.748**	-0.166^{NS}	0.418^{NS}	0.699**	1.000



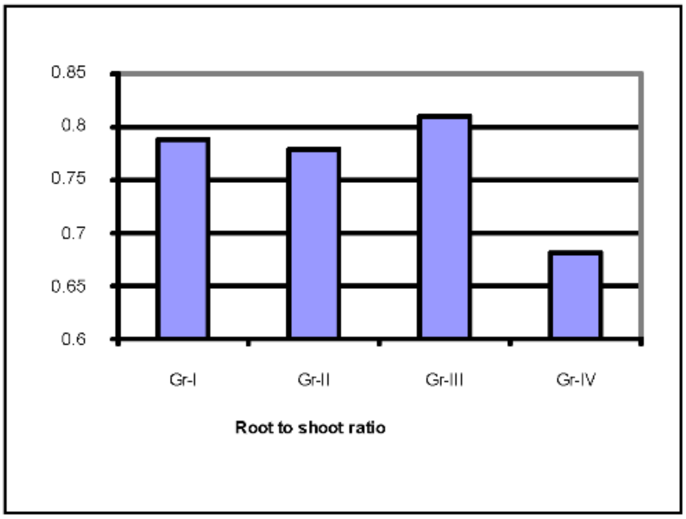


Fig 1 & 2: Classification of grafts based on root weight and root to shoot ratio

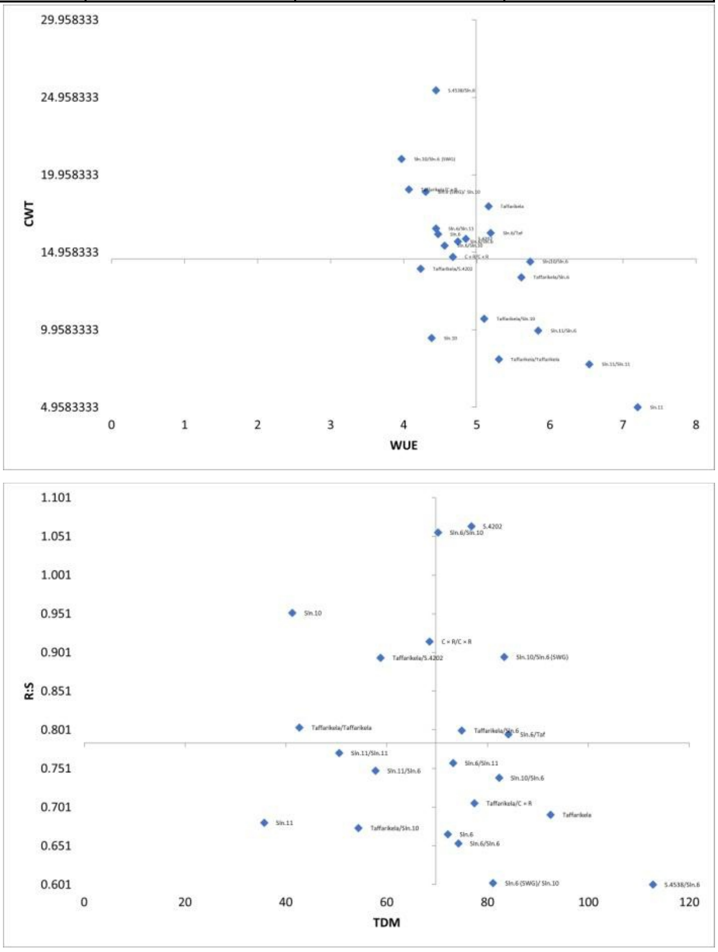


Fig 3: Normal Z distribution of clustering the grafts cumulative water transpiration (CWT) vs water use efficiency (WUE) and root: shoot ratio vs total dry matter (TDM)

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