Original Research Article

Comparison of stalk volume by water displacement method and calculation method for stalk weight determination and its relevance to single cane weight in sugarcane clones

Abstract

The stalk volume by water displacement method (SVWM) and stalk volume by calculation method (SVCM) were compared for the efficacy of stalk weight determination in sugarcane clones. Results from both methods were similar and a highly significant relationship was found between the two methods (r^2 = 0.9092, P < 0.0001). Both protocols provide consistent stalk volume measurements; however, the stalk volume calculation method is preferred for its quick evaluation of a large number of stalks in a rapid way besides its non-destructive nature. The calculation method saved more than 70 seconds for each sample.The stalk weight has shown a significant correlation of r=0.71***, 0.83***, 0.94***, 0.99*** with SH, CD, SVCM and SVWM respectively, while the stalk density (SD) was observed with a negative correlation (r=-0.27^{ns}) with stalk weight. The stalk volume-based predicted stalk weight estimation was observed with high degree of correlation (>0.88***) with the original single cane weight by both the studied methods, thus, measurements of stalk volume based on plant height and stem circumference can provide simple, rapid, non-destructive field phenotyping of single cane weight in sugarcane crop.

Comment [D1]: Please add the objective of carrying out the research Recommendation

1. Introduction

Sugarcane is the second most important commercial crop in Indian agriculture and it's being cultivated nearly 5 Mha, with productivity of 82t/ha respectively. Sugar, jaggery, molasses, bagasse etc. were the high-demand products of sugarcane, and being a C_4 crop it grows well under arid hot weather compared to other C_3 crops and the weather variability, rising human population, land constraints, etc has led the scientific forum [1] for the identification of several management practices including utilization of location-specific tolerant varieties for sustaining sugarcane productivity under changing climate scenario. The ICAR-SBI has developed more than 2800 Co canes and they are of massive importance for their high variability and suitability against biotic and abiotic stress. In order to screen these sugarcane clones for higher productivity high throughput tools are highly essential, and estimation of stalk volume by calculation method has been reported as one of the traits for predicting stalk weight/yield in a non-destructive way [2].

In sugarcane, the yield is mainly determined by number of millable clones (NMC) and stalk weight or single cane weight (SCW). The weight per unit area of land harvested is considered as the yield of a field crop [3] and the yield is considered as most complex feature which includes integration of many traits and their interaction with environment and finally the source sink relation resulting in economic yield. In most of the crops the grains are considered as yield, while in sugarcane the stem portion is known for its sugar extraction for

industrial purpose. In sugarcane among the various studied parameters, the leaf area index (LAI) has been reported to have better correlation with cane yield [4].

Nowadays non-destructive method of plant phenotyping [5] for accelerated plant breeding is being widely discussed among various research forum, and particularly the traits related/correlated to the crop yield is of high importance for the reason of their practical benefits and profitability which is one of the major focus rather than of mere basic study. Among the various crop, the canopy coverage is being discussed for measuring canopy volume and its association with crop yield, while in sugarcane, the stalk portion is the primary/economic product of the crop, and identification of stalk fresh weight is essential to predict the crop yield. Mostly the sugarcane stalk is considered cylindrical in shape and the formula for calculating the cylinder volume [6,7] is used for determining the stalk volume of sugarcane [2] which has a high correlation with crop yield, and very few reports are available in this type of findings. Vasantha et al. [2] have reported the association of stalk volume by calculation method with the stalk weight. A comparison of the water displacement method and stalk volume calculation method for stalk weight determination in sugarcane clones has not yet been established till data and very few reports are available for the non-destructive calculation of the fresh weight of sugarcane stalk, and the present investigation was carried out with the objective (i) to compare the water displacement method and stalk volume calculation method for the stalk volume determination and (ii) to find the association between the stalk volume with stalk weight for the estimation of the single cane weight of sugarcane crop rapidly in non-destructive way.

2. Materials and methods

In the present study, 32 single cane of 10-month duration sugarcane was collected from the sugarcane experimental plot at ICAR-Sugarcane Breeding Institute, Coimbatore (11° 0' 34" N, 76° 55' 2" E, 430 m above mean sea level), Tamil Nadu, India maintained scientifically by following the recommended cultural package of practices. The 32 single canes from various sugarcane clones were earmarked, and the stalk volume was determined based on the calculation method, and after that the stalk was collected, detrashed and the stalk volume was determined by the water displacement method.The plant height was measured from the base to the top most visible transverse mark on the 300^{th} day after planting (DAP) using a measuring tape and the shoot thickness with a digital vernier caliper (Mitutoyo) [2]**.** Also, the single cane weight of 73 sugarcane clones (10 months old) was used to find the association of SCW with the stalk volume derived through the calculation method.

2.1 Stalk volume determination based on calculation method

Mostly the sugarcane stalk is considered cylindrical in shape (Figure 2) and the stalk volume was determined by following the equation:

$$Stalk\ volume = \pi r^2 h \quad \text{-------(1)}$$

Where r is the radius (cm) of the stalk measured through vernier calipers and h is the height (cm) of the stalk determined using a standard measuring tape. The calculated stalk volume is mentioned in the form of cm^3.

2.2 Stalk volume determination based on the water displacement method

Traditionally the volume of an object can be measured using the water displacement method based on Archimedes' principle [8] i.e When an object is partially or wholly immersed in a fluid it experiences an upthrust force equal to the weight of the fluid displaced by the object. The same stalk of the sugarcane used for the calculation method was utilized for the determination of the stalk volume. The weighted canes/stalks were cut into 4-5 pieces and were immersed/submerged until all surfaces are submerged in the water filled in a container having graduation markings. The stalk volume was calculated as follows:
Stalk volume = volume of the water after submerging the stalks into the container – volume of the water before submerging the stalks. A submerged sugarcane stalk displaces a volume of liquid equal to the volume of the stalk (Figure 1). The volume of displaced water was considered as the volume of the object and one milliliter (1 mL) of water has a volume of 1 cubic centimeter ($1cm^3$). Since the stalks of the sugarcane are non-porous the stalk volume measured was of a high degree of accuracy.

2.3 Statistical analysis

In order to compare the stalk volume by water displacement method (SVWM) with stalk volume by calculation method (SVCM) a paired t-test was conducted to determine whether there exists statistical evidence that the associated population means are significantly different or the same. Regression analysis was carried out between stalk volume by water displacement method with stalk volume by calculation method on the dependent sample and the corresponding slope coefficient or regression coefficient (β), and significance was done following the "paired t" test @ 0.05% probability. Also, another regression analysis was carried out between the two studied methods with the stalk weight. Ordinary least squares (OLS) regression model was used where the conditional mean of the response variable given the predictor variables. A correlation diagram displaying the correlation (correlation coefficient value (r) between -1 to 1, with a value of -1 meaning a total negative linear correlation, 0 being no correlation, and + 1 sense a total positive correlation) between the studied parameters along with the P-value was done through R-studio Software. All the statistical analysis was done through JMP 9.2 version software.Meantime used for measuring the stalk volume by water displacement method and calculation methods were analyzed by mean comparison followed by t-test and tukey test.

3. Results and discussion

3.1 Comparison of stalk volume by water displacement method with stalk volume by calculation method through paired t-test

In order to compare the stalk volume by water displacement method (SVWM) with stalk volume by calculation method (SVCM) a paired t-test was conducted using the "R" Program and the results are shown in Table 1. The results of the analysis indicate that the mean for the SVWM is 992, and for the SVCM it is 1177. The average difference between the paired

SVWM and SVCM scores is -185.00. the p-value is less than the significance level (0.05), favours the H1: the difference does not equal zero. Because the p-value for the paired sample t-test is less than the standard significance level of 0.05, we can reject the null hypothesis. Our sample data support the notion that the average paired difference does not equal zero. Specifically, the SVWM and SVCM methods are statistically different. The confidence interval estimates (-185.00) that the actual population difference between the SVWM and SVCM is likely between -234.86 and -135.14 confident interval and it can be concluded that both the methods are significantly different. The negative values reflect the fact that the SVWM has a lower mean than the SVCM (*i.e.*, SVWM – SVCM < 0). The confidence interval excludes zero (no difference between the paired samples) as a likely value, so we can conclude that the population difference does not equal zero. These results are in accordance with the [9] report on the comparison of water displacement, and WinRHIZO software for plant root parameter assessment in Bermuda grass.

3.2 Correlation between the studied stalk parameters

The correlation between the various stalk traits *viz.*, Stalk height (SH), Cane diameter (CD), SVWM, SVCM, and stalk weight (SW) are shown in figure 3. The stalk weight has shown a significant correlation of r=0.71***, 0.83***, 0.94***, 0.99*** with SH, CD, SVCM and SVWM respectively. And, the Stalk density (SD) was observed with a negative correlation ($r=-0.27^{ns}$) with SW. In sugarcane variety development programs, where increased cane yield is the primary goal, emphasis should be placed on selecting the major yield components: stalk number, diameter, and length [7]**.** They also reported similar correlations between the plant height, number of tillers, cane diameter, and cane yield in sugarcane. It was reported that the number of tillers and plant height at six months after planting is highly correlated with canopy cover (rg = 0.72) and canopy height (rg = 0.69), respectively and our results corroborate with the previous study of [10]which reported that early bio-mass had a high genetic correlation with unmanned aerial vehicle (UAV)-derived canopy height (0.810). Raman et al. [11] reported that the number of stalks per stool was a major yield contributing factor followed by height and cane diameter [12]**.** Further, million et al., 2008 et al also reported a significant linear relationship between stalk height and stalk weight in sugarcane crop, and our study also indicated similar results. Recently, Vasantha et al. [2] reported stalk volume (calculation method) in sugarcane with a significantly better correlation with cane yield or stalk weight thus confirming our findings.

3.3 Comparison of the association of stalk volume by water displacement method with stalk volume by calculation method on the dependent sample through regression

The results revealed that there exists a close relationship between the stalk volume by water displacement method, with stalk volume by calculation method (Figure 4). The SVWM and SVCM show a straight-line association on a scatter diagram and confirm the perfect linear regression ($Y=44.66+0.81x$, $R^2=0.9092$, P value=<2.2e-16) between the two variables. The straight regression line showing the relation confirms the better relationship between the studied variables and the results revealed that the linear regression relationship between the two variables is extremely consistent. The results demonstrate that the SVCM is superior to

SVWM and it can be used for stalk weight calculation instead of SVWM (in which samples have to be destructed and is also time-consuming compared to SVCM). These results corroborate with the report on the comparison of water displacement [7], and calculation method for the stalk volume in sugarcane.

3.4 Association of stalk volume by water displacement method, with stalk weight

The results revealed that there exists a close relationship between the stalk volume by water displacement method, with stalk weight (Figure 5). The stalk weight is a dependent variable (cause) and the stalk volume is an independent variable (effect) in the study. The stalk weight and stalk volume show a straight-line association on a scatter diagram and confirm the perfect linear regression between the two variables. The straight regression line showing the relation confirms the better relationship between the studied variables and the results revealed that the linear regression relationship between the two variables is extremely consistent, and their slope is 0.98 which explains that for every 1-unit change in explanatory variable x (Stalk volume by displacement method) there is corresponding 0.98-unit change occurs in response variable y (stalk weight). This indicated that the stalk volume determined through the water displacement method can be used to assess the stalk weight accurately. The paired t-test also revealed that there exists significant correlation (p-value: <2.2e-16) between the stalk volume (water displacement) and stalk weight. Further, the coefficient of determination or R^2 which measures the goodness of fit of the present model also indicated with better approximation (R^2=0.98***) of regression line with the actual data or in other words the R^2 of 0.98 means that our predictor variable (stalk volume by displacement method) explain 98% of the variance in the output (stalk weight) we are trying to predict. These results are in agreement with the report on the for plant root parameter assessment in Bermuda grass [9].

3.5 Association of stalk volume by calculation method, with stalk weight

The results revealed that there exists a close relationship between the stalk volume by calculation method, with stalk weight (Figure 6). The straight regression line showing the relation confirms the better relationship between the studied variables and the results revealed that the linear regression relationship between the two variables is extremely consistent, and their slope is 0.79 which explains that for every 1-unit change in explanatory variable 'X' (Stalk volume by calculation method) there is corresponding 0.79-unit change occurs in response variable y (stalk weight). This indicated that the stalk volume determined through the water displacement method can be used to assess the stalk weight accurately. The paired t-test also revealed that there exists significant correlation (p-value: <7.604e-16) between the stalk volume (water displacement) and stalk weight. Further the coefficient of determination or R^2 which measures the goodness of fit of the present model also indicated with better approximation (R^2=0.88***) of regression line with the actual data or in other words the R^2 of 0.88 means that our predictor variable (stalk volume by displacement method) explain 88% of the variance in the output (stalk weight) we are trying to predict. These results are in agreement with the [2] report on the stalk volume in sugarcane where they have reported significant correlation between the stalk volume and cane yield.

3.6 Meantime used for measuring the stalk volume by water displacement method and calculation methods

The average times required to measure a sample using each method were recorded (i.e., 78 s for water displacement and 6 s for calculation method (Figure 7). The calculation method saved more than 70 seconds for each sample. Time data analyzed using a simple "t" test revealed that the means of two independent groups are significantly different and further it has been confirmed by the post hoc Tukey test. These results are in agreement with the report on the plant root parameter assessment in Bermuda grass [9] with two different methods.

3.7 Prediction of single cane weight (SCW) by stalk volume

Based on the stalk volume the simple regression analysis has revealed the prediction of cane stalk weight by SVWM and SVCM as per the equation 2 mentioned below:

$$SCW = (0.064 + 0.0009x) \text{--------} (2)$$

This simple equation suggests the usefulness of the stalk volume trait in forecasting stalk weight in sugarcane in a rapid and accurate way. The results revealed that the stalk volume calculated based on the calculation method has a significant correlation (R^2=0.913***) with the stalk weight of the crop. The regression analysis indicated that the stalk volume determined through the calculation method can be used to assess the cane yield accurately. In sugarcane-breeding programs where increased cane yield is the primary goal, emphasis should be placed on selecting major yield components *viz*., number of millable canes, stalk weight, stalk diameter, and length [7]and they also reported significant correlation between the stalk weight and stalk volume. Our results are also in agreement with [2]in sugarcane crop.

4. Conclusion

The present investigation revealed thatthe stalk volume by water displacement method (SVWM) and stalk volume by calculation method (SVCM) were compared for the efficacy of stalk weight determination in sugarcane clones and the results from both methods were similar and highly significant relationship was found between the two methods (r^2 = 0.9092, P < 0.0001). The stalk volume calculation method is preferred for its quick evaluation of a large number of stalks in a rapid way besides its non-destructive nature. The calculation method saved more than 70 seconds for each sample.The stalk weight has shown a significant correlation of r=0.71***, 0.83***, 0.94***, 0.99*** with SH, CD, SVCM, and SVWM respectively, while the stalk density (SD) was observed with a negative correlation (r=-0.27^{ns}) with stalk weight. The stalk volume-based projected stalk weight estimation was observed with high degree of correlation (>0.88***) with the original single cane weight by both the studied methods, thus, measurements of stalk volume based on plant height and stem circumference can provide simple, rapid, non-destructive field phenotyping of single cane weight in sugarcane crop.In sugarcane, the yield is mainly determined by number of millable clones (NMC) and stalk weight or single cane weight (SCW) and the stalk volume-based predicted stalk weight estimation had a significant positive correlation with the original SCW thus signifying the importance of SVCM for determining the SCW in a non-destructive way.

Comment [D2]: Add up the recommendation.

Reference

1. Habib-ur-Rahman M, Ahmad A, Raza A, Hasnain MU, Alharby HF, Alzahrani YMet al.Impact of climate change on agricultural production; Issues, challenges, and opportunities in Asia. Front Plant Sci. 2022;13:925548. doi: 10.3389/fpls.2022.925548
2. Vasantha S, Raja AK, Vengavasi K, Tayade AS, Shareef A, Govindakurup H. Sugarcane stalktraits for highthroughputphenotyping in restrictedirrigationregimes. Sugar Tech. 2023;3:1-9. doi: 10.1007/s12355-023-01242-7.
3. Watson DJ. The physiologicalbasis of variation in yield. Adv Agron. 1952;4:101-45. doi: 10.1016/S0065-2113(08)60307-7
4. IrvineJE. Relations of photosyntheticrates and leafcanopy characters to sugarcaneyield. Crop Sci.1975;15(5):671-6. doi: 10.2135/cropsci1975.0011183X001500050017x.
5. Meena MR, Appunu C, Arun Kumar R, Manimekalai R, Vasantha S, Krishnappa Get al.Recent advances in sugarcane genomics, physiology, and phenomics for superior agronomic traits. Front Genet. 2022;13:854936. doi: 10.3389/fgene.2022.854936.
6. Dillewijn C.V. Botany of sugarcane. The chronicabotanicaco.Waltham, MA: Book Department, 1962; p. 61.
7. MillerJD, James NI. The influence of stalk density on cane yield. Proceedings of thesugarcanetechnologists. 1974;15:177-84.
8. Siswantoro J, Prabuwono AS, Abdulah A. Volume measurement of food product with irregular shape using computer vision and Monte Carlo method: a framework. Procedia Technol. 2013;1(11):764-70. doi: 10.1016/j.protcy.2013.12.256
9. PangW, CrowWT, LucJE, McSorleyR, Giblin-DavisRM, KenworthyKEet al.Comparison of water displacement and WinRHIZO software for plant root parameter assessment. Plant Dis. 2011;95(10):1308-10. doi: 10.1094/PDIS-01-11-0026
10. NatarajanS, BasnayakeJ, WeiX, LakshmananP. High-throughputphenotyping of indirecttraits for early-stageselection in sugarcanebreeding. Remote Sens. 2019;11(24):2952. doi: 10.3390/rs11242952.
11. Raman K, Bhat SR, Tripathi BK. (year)Ratooning ability of sugarcane genotypes under late harvest conditions. Indian Sugar. 1985;35:445-8.
12. Million F, Mohammed H, Tena E.(year)Correlation of traits among cane yield and its component in sugarcane (*Saccharum*spp.) genotypes at metahara sugar estate. IntJAdvResBiolSci.2018;5(11):56-61.

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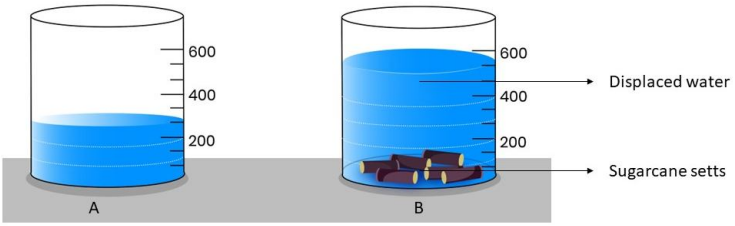

Figure 1.Stalk volume determination through water displacement method (A) Large beakers initial water level (B) Large beakers with sugarcane setts immersed and the water displaced.

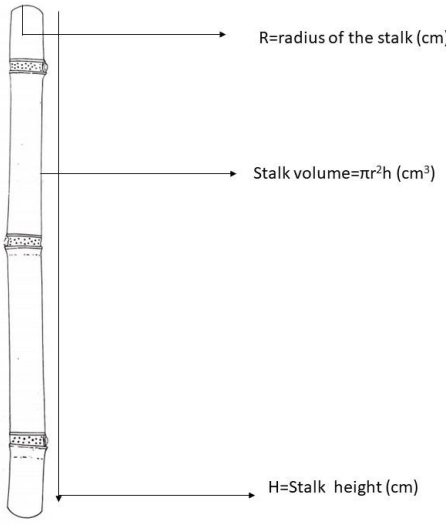

Figure 2. Stalk volume determination through calculation method

Table 1. Comparison of stalk volume in sugarcane by water displacement method and calculation method

S.no	Stalk volume by water displacement method (cm^3)	Stalk volume by calculation method (cm^3)
1	630.00	725.97
2	610.00	700.69
3	390.00	595.33
4	470.00	669.63
5	1210.00	1411.02
6	1890.00	2272.97
7	1050.00	1480.44
8	1110.00	1429.08
9	700.00	826.96
10	680.00	688.27
11	320.00	454.90
12	820.00	1117.93
13	1055.00	1159.65
14	1630.00	1986.66
15	1280.00	1773.65
16	701.00	814.77
17	850.00	1106.09
18	840.00	1085.95
19	810.00	661.53
20	1530.00	1559.17
21	1240.00	1552.89
22	910.00	947.95
23	1360.00	1427.32
24	960.00	1069.26
25	1195.00	1328.00
26	1620.00	1897.00
27	940.00	1115.69
28	1200.00	1188.88
29	970.00	1186.67
30	1020.00	1254.90
31	940.00	1238.12
32	820.00	943.79
Mean	**992.22**	**1177.22**
	Paired t-test results:	
	T value: -7.5679	
	Df=31	
	P value=1.569e-08	
	Confidence intervals: -234.6 , -135.1	
	Sample estimates: **Mean difference:** -185.0	
	Null hypothesis (H_o: $\mu_1 = \mu_2$) and **alternate hypothesis** (H_o: $\mu_1 \neq \mu_2$)	

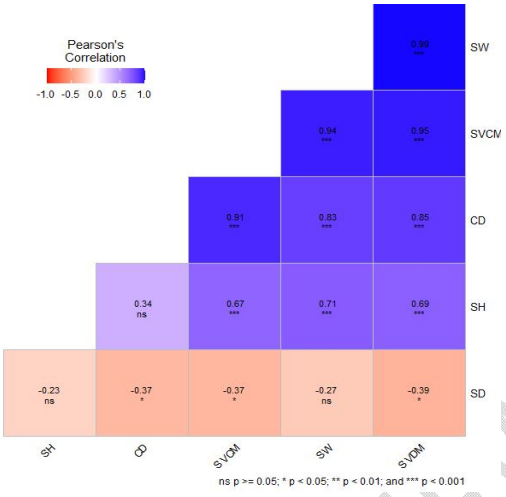

Figure 3.Correlation between various stalk traits viz., SH, CD, SVCM, SW, SVDM. ** denotes significant at 1%, * denotes significant at 5%, and ns denotes non-significant. The intensity of the colour indicates the strength of the correlation.

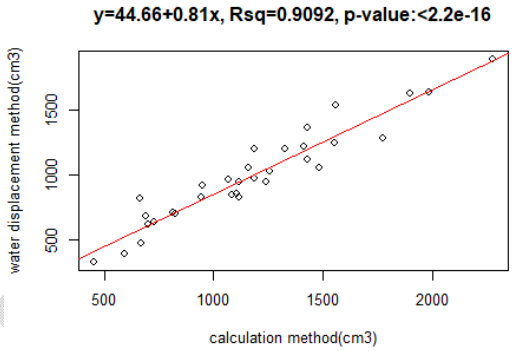

Figure 4. Association of stalk volume by calculation method with stalk volume by water displacement method

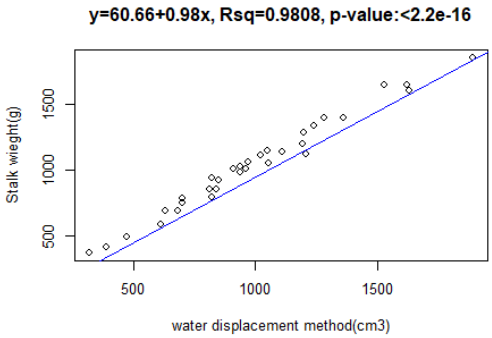

Figure 5. Association of stalk volume by water displacement method with stalk weight

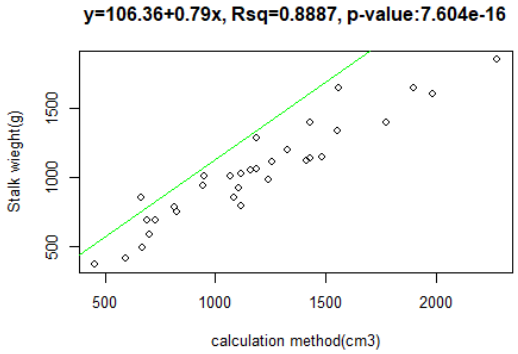

Figure 6. Association of stalk volume by calculation method with stalk weight

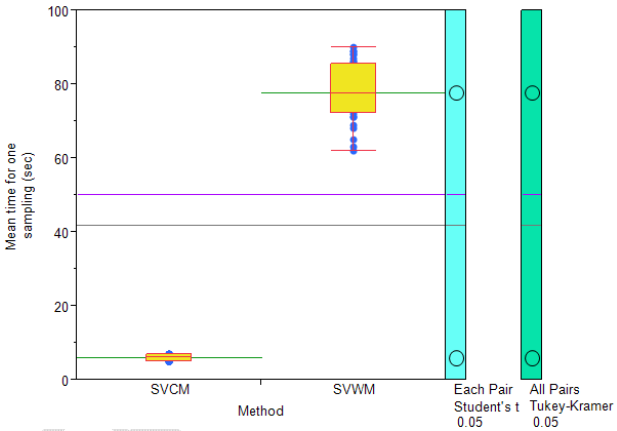

Figure 7. Meantime used for measuring the stalk volume by water displacement method and calculation methods

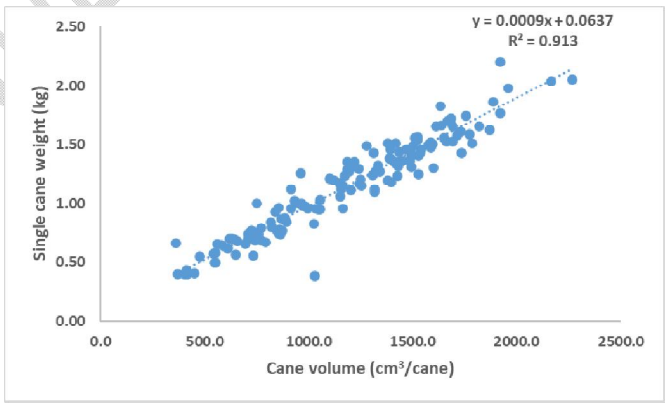

Figure 8. Association between the stalk volume by calculation method and single cane weight