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Development of Linear Model for Leaf Area Measurement to Screen Potential Leaf Material for Herbal Drug in *Putranjiva roxburghii.*

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Abstract: *Leaf area is a key variable in the study of physiology , horticulture and crop science It is major parameter in plant modelling studies, to develop physiological model in plant breeding .Leaf area is one of the important growth factor for plants especially in medicinal plants and lack of accurate model is a limitation for calculating LA. The plant Putranjiva roxburghii (Medicinal plant) was selected and the leaves of these plants were used for development of linear model for leaf area using Counting grid method. Leaf samples were randomly selected from the lower, middle and upper parts of the plant with different sizes of Leaves. Length, width, L^2,W^2, product of these dimensions and leaf fresh and dry weights and their water content of 25 leaf samples were assessed and compared with actual leaf area measured by graph tracing method, to test their accuracy and reliability using Y = a + bX model. There was a highly significant correlation (r = 0.9599 to 0.9967 for Putranjiva between actual leaf area and the corresponding leaf length, width, L^2, W^2, product of these dimensions and leaf fresh and dry weights. Regression analyses of LA versus FW, DW, L, W, L^2, W^2 and the products of these dimension revealed several models that could be used for estimating the area of individual leaf of both plants. Among the models, one based on length and width dimension (LA = a + bL*W) r = 0.9967, R^2 = 0.9936 (Putranjiva) was the most accurate. To validate this model, actual leaf area of 25 leaf samples obtained by Count grid method was compared with leaf area estimated by the this model. The leaf area estimated by the models strongly agreed with the measured value of leaf area as evident from high value of R^2 (0.9978) for Putranjiva. The validation of the models indicates that model (LA = a + bL*W) was accurate and reliable to determine the leaf area of the plant and therefore it would be very useful for field workers dealing with large samples. And* ***the development of linear models for Putranjiva roxburghii is firstly reported in Gujarat during this study.***

Key Words: *Medicinal plants, Putranjiva roxburghii, Leaf area measurement, count grid method*

1. INTRODUCTION:

Putranjiva roxburghii , also known as *Drypetes roxburghii* belongs to Euphorbiaceae family. It is abundantly grows in Asian tropical regions (Haldar *et al.*, 2009)[1]. *Putranjiva roxburghii* is a tree which normally grows upto 20-22 m height. It is whitish in colour when young and turns to dusky grey colour at maturity with straight pores and pendulous branching. Roxburgh (1832)[2] explained the name of the tree "putranjiva", (Sanskrit) Putra meaning a son and jeeva means life. Plant's parts possesses anti-inflammatory, antioxidant, antipyretic, antinociceptive and antimicrobial properties. It contains phytochemicals like Beta amyrin and it's esters, putrone, putrol, putranjivic acid, methyl putranjivate, stigmasterol and hydrocarbons, triterpene roxburghonic acid and biflavones. Leaves are useful to cure cold, fever , rheumatism , burning sensations , itching , haemorrhoid, muscle twisting(Boonyaprapat N ,Chokechaicharoeporn A., 1999)[3]Singh et al. (2010) reported the use of leaf juice in washing inflected eyes, cold, dysentery, aphrodisiac , fever, stimulant and spermopoietic[4].The leaves of putranjiva are the commercially important plant part, and by estimating leaf area (LA),Production could be predicted which is useful for herbal drug preparation(Daramola et al.,2018)[5].Growth of plant depends upon the leaf area, number of leaf & stem, stem width and plant height. Leaf area plays a vital role in plant growth analysis and photosynthesis. Leaf area can be measured using different destructive and non-destructive methods. For studying primary production in plants, plant physiologists require leaf area measurements. [(Sestak *et al.*, 1971)[6]; (Tieszen, 1982)[7];(Bleasdale,1984)[8], provided an elaborative description of the most common methodology available till date that includes counting squares on millimeter graph paper, handplanimetry, the gravimetric method, dot counting, photoelectric planimetry, air flow, linear measurements of leaves, leaf weighing, detached leaf counting, and the rating method.

An appropriate method of leaf area measurement in these plant species must not reduce or damage the leaves, because they are very important medicinal plants. The use of regression models can provide simple, quick, accurate, reliable, inexpensive, rapid, and non-destructive alternative method for estimating leaf area to within 0.05 accuracy (Raju *et al*., 1991[9]; Uzun and Celik, 1999)[10]. Such models eliminate the need for leaf area meters and save time

(NeSmith, 1992)[11]. And also are useful in studying plant activities, which requires a non-destructive method of leaf area measurement and also when the number of available plants is limited (Pinto *et al*., 2004)[12]. The procedure of this method involves measuring lengths, widths and areas of samples of leaf and then calculating several regression equations to estimate areas of subsequent leaf samples (Pouono *et al*., 1990[13]; Pinto *et al*., 2004)[12], but the importance of developing models as a rapid measurement of leaf area in agronomic and physiological studies is well known and established for other crops in the literature (Bhatt and Chanda, 2003[14]; Lu *et al*., 2004;[15] Gamper, 2005[16]) such models have not yet been established for estimating the leaf area of *Putranjiva roxburghii* in Gujarat. This study was therefore undertaken with the objective of developing linear model for leaf area measurement of this plant using the best matching regression equation of intact leaves *Putranjiva roxburghii* using functions between plant LA and plant vegetative characteristics.

2. MATERIALS AND METHOD:

2.1. Collection of plant materials:

Putranjiva roxburghii leaves were collected from the campus of Gujarat University, Ahmedabad, Gujarat. Leaf samples(25) were randomly selected from the lower, middle and upper parts of the plant with different sizes of Leaves.

2.2. Measurements of growth parameters:

Growth was measured in the terms of fresh weight, dry weight, water content, length, and width and leaf area. For the measurement of fresh and dry weights, freshly harvested smaller to larger sizes of leaves were taken. Freshly seperated leaves were weighed before and after sun drying to a constant weight for 6-7 days to obtain the data on fresh and dry weights. Water contents of each stage were determined by difference in fresh and dry weight (gm). The length (L, in cm), width (W, in cm) and area (A, in cm^2) of individual leaves were determined. Leaf length (L) was measured from lamina tip to the point of intersection of the lamina and stem and width (W) were measured from tip to tip between the widest lamina with a simple ruler. Leaf area of 25 leaves were measured by counting grid square method according to Stewart and Dwyer, 1999[17] with some modifications. The actual leaf area (dependent variable) was then regressed on their linear measurements (independent variables), including, L, W, L^2, W^2, the products of these dimension (L+W, L×W, L/W, $L^2 \times W^2$, $L^2 + W^2$) and also with dry weight, fresh weight and water contents of leaves to identify appropriate functions for use in models estimating leaf area of the plant. The values of the coefficients (b) and constants (a) were also reported. The estimated LA was determined by fitting the equation and the final model was selected based on the combination of the highest coefficient of determination (R^2) and correlation coefficient (r).

Figure 1. Leaves of *Putranjiva roxburghii*

2.3. Statistical Analysis:

All collected data were subjected to correlation analysis like linear correlations between leaf area and planimetric or gravimetric parameters using Excel software (MS office, Microsoft).

3. RESULT AND DISCUSSION:

The average, minimum, maximum and standard deviation for leaf area, length(L), width(W), L^2, W^2 and the products of these dimension($L + W$, $L \times W$, $L^2 \times W^2$, $L^2 + W^2$) and fresh and dry weights and their water contents of selected individual leaves of the plant of different sampling sizes is shown in table.1 (*Putranjiva roxburghii)* From ***Putranjiva roxburghii*** , 25 leaves were taken for the area measurement by using counting grid square method which showed the range between 3.9 to 34.28 cm^2 areas for the leaves tested. Minimum leaf area was observed 3.9 cm^2/leaf whereas, maximum leaf area was 34.28 cm^2/leaf. Each individual leaf was weighed before and after sun drying for constant weight. DW ranged between 0.014 to 0.296 mg/ leaf. WC ranged between 0.032 to 0.272 mg/ leaf and FW ranged between 0.046 to 0.568 mg/leaf. The obtained DW, WC and FW were plotted against respective leaf area showed linear relationship. Difference between minimum and maximum values were observed for each measured variable of

planimetric parameter (3.4 cm ≤ length ≤ 12.4 cm, 1.5 cm ≤ width ≤ 4 cm, 11.56 cm² ≤ length² ≤ 153.76, 2.25 cm² ≤ width² ≤ 16, 5.2 cm ≤ length + width ≤ 16.1, 6.12 cm² ≤ length × width ≤ 45.88cm², 14.8 cm² ≤ length² + width² ≤ 167.45, 37.45 cm² ≤ length² × width² ≤ 2104.97)in leaves which are used for the mathematical generation of models of leaf area estimation(Table.1). The obtained L, L², W, W²,L+W,L²+W², L×W, L²×W² were plotted against respective leaf area showed linear relationship. There was a highly significant positive correlation between actual leaf area (LA) and leaf length and leaf width and functions of these measurements described by using Y=a + bX(r=0.9599 to 0.9967) (Table.2). Similarly, significant correlation was observed between LA and leaf fresh and dry weights and their water contents which are described by Y= a + bX(r= 0.9902,0.9803 and 0.9636 respectively).

Correlation coefficient(r) and coefficient of determination (R^2) of the various models are shown in table.2. Based on selection criteria (highest R^2 and r) we selected the best model for estimating leaf area of ***Putranjiva roxburghii***. All models produced a coefficient of determination (R^2) equal to or greater than 0.9216. From the result of this study, models 1,2,3,4,6,7,8,10,11 are less acceptable for estimating leaf area of ***Putranjiva roxburghii*** because of their lower coefficient of determination (R^2=0.9216,0.9296,0.9474,0.9312,0.9544,0.9612,0.9634,0.9612 and 0.9285 respectively) while model 5 and 9 are more acceptable for estimating leaf area of ***Putranjiva roxburghii*** because of their higher coefficient of determination (R^2= 0.9936 and 0.9805 respectively) (Table.2)

3.1. Model validation for *Putranjiva roxburghii:*

To validate the best model, different size of 25 leaves of ***Putranjiva roxburghii*** were taken to compare leaf area estimated by the linear model Y = a + bX with actual leaf area as determined by graph tracing method. Actual leaf area, leaf length and leaf width were determined by the previously described procedure. Leaf area of individual leaves was predicted using the best model from the calibration experiment and was compared with the actual leaf area. Regression analysis was conducted and comparisons were made between measured versus calculated leaf area of different size of leaves of by using the best model (LA = a + bL×W) where LA is individual leaf area (cm²), L is the leaf length (cm) and W is the leaf width (cm).

The leaf area estimated by this model strongly agreed with the measured value of leaf area of the leaves as evident from high value of R^2 (0.9978) (Figures 6). The validation of the model indicates that leaf area of ***Putranjiva roxburghii*** could be measured rapidly and accurately by using this linear developed model.

Table 1.Descriptive statistics of the leaf parameters measured during the experiment of *Putranjiva roxburghii* from Gujarat.

	Leaf Area (cm^2)	Length (cm)	Width (cm)	L^2 (cm^2)	W^2 (cm^2)	L + W (cm)	L×W (cm)	$L2×W^2$ (cm^2)	L2 + W2 (cm^2)	FW (g)	DW (g)	WC (g)
Min	3.9	3.4	1.5	11.56	2.25	5.2	6.12	37.45	14.8	0.046	0.014	0.032
Max	34.28	12.4	4	153.76	16	16.1	45.88	2104.97	167.45	0.568	0.196	0.272
Mean	16.2	7.77	2.72	67.15	7.95	10.5	22.89	679.49	75.11	0.266	0.115	0.150
SD	9.39	2.65	0.73	40.86	4.14	3.33	12.72	650..63	44.60	0.163	0.082	0.083
Var	88.25	7.03	0.53	1670.35	17.20	11.08	162.02	423329	1989.69	0.026	0.006	0.007

Table 2.Fitted coefficient (b), constant (a), correlation coefficient (r) and coefficients of determination (r^2) values of the models used to estimate *Putranjiva roxburghii* leaf area (LA) of single leaves from length (L) and width (W) measurements.

		Fitted coefficient and constant			
No.	**Model tested**	**a**	**b**	**R^2**	**r**
1	LA = a + bL	-10.227	3.4003	0.9216	0.9599
2	LA = a + bW	-17.49	12.35	0.9296	0.9641
3	LA = a + bL^2	1.1744	0.2237	0.9474	0.9733
4	LA = a + bW^2	-1.1954	2.1858	0.9312	0.9649

5	**LA = a + bLW**	-0.64	0.7357	0.9936	0.9967
6	LA = a + b (L + W)	-12.739	2.7561	0.9544	0.9769
7	LA = a + b ($L^2 + W^2$)	6.5808	0.0142	0.9612	0.9804
8	LA = a + bL^2W^2	0.6718	0.2067	0.9634	0.9815
9	LA = a + bFW	1.03	56.97	0.9805	0.9902
10	LA = a + bDW	3.3013	111.7	0.9612	0.9803
11	LA = a + bWC	-0.07	107.89	0.9285	0.9636

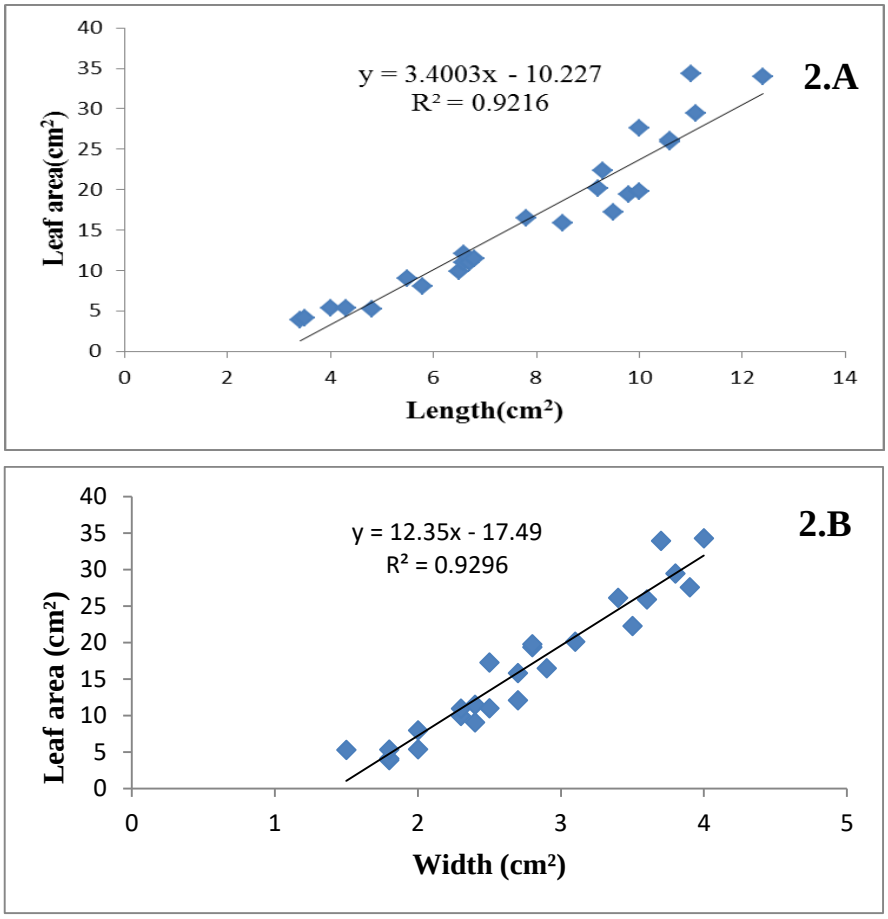
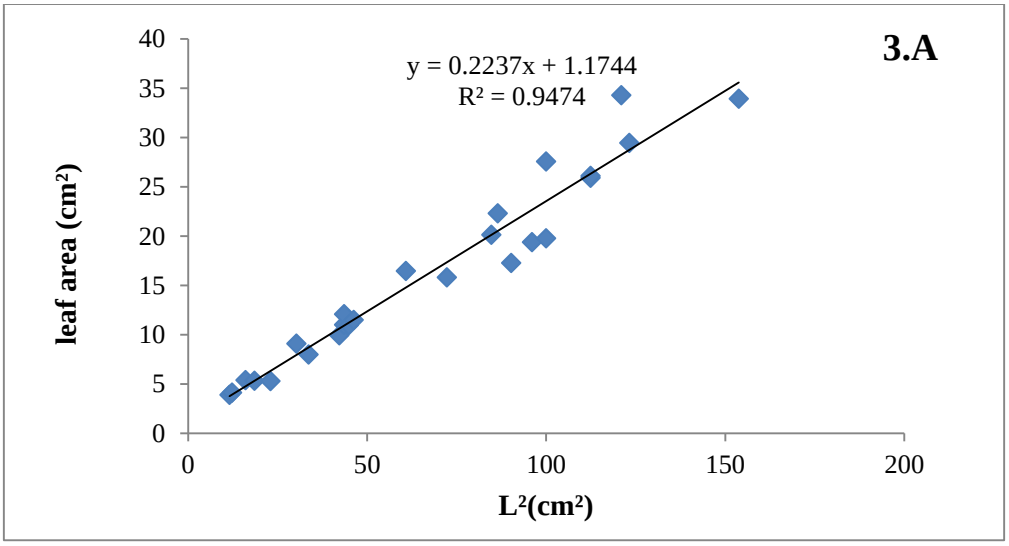


Figure 2: A-Relationship between leaf area (LA) and leaf length (L) and B- between leaf area (LA) and leaf width (W) of single leaves of *Putranjiva roxburghii*



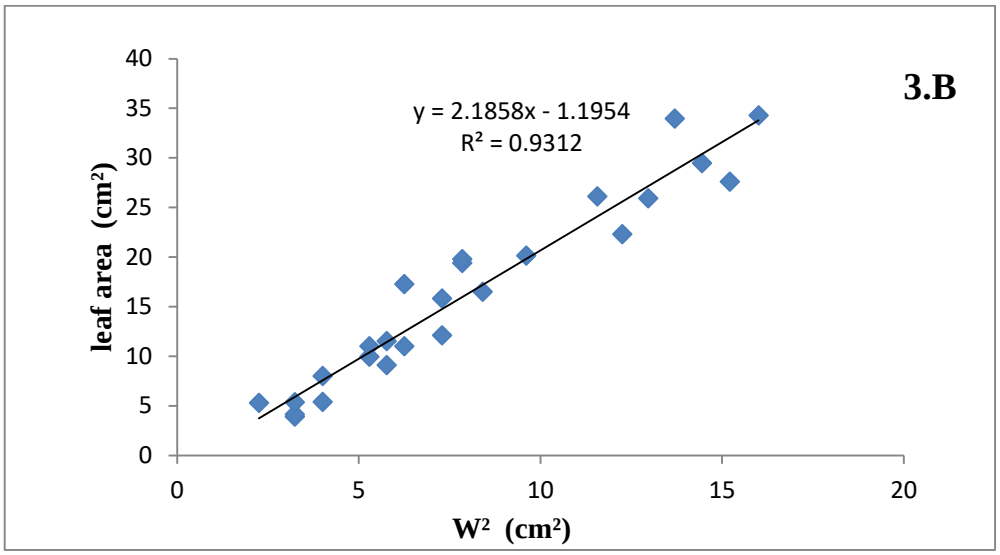


Figure 3: A - Relationship between leaf area (LA) and leaf length (L^2) and B- between (LA) and leaf width (W^2) of single leaves of *Putranjiva roxburghii*

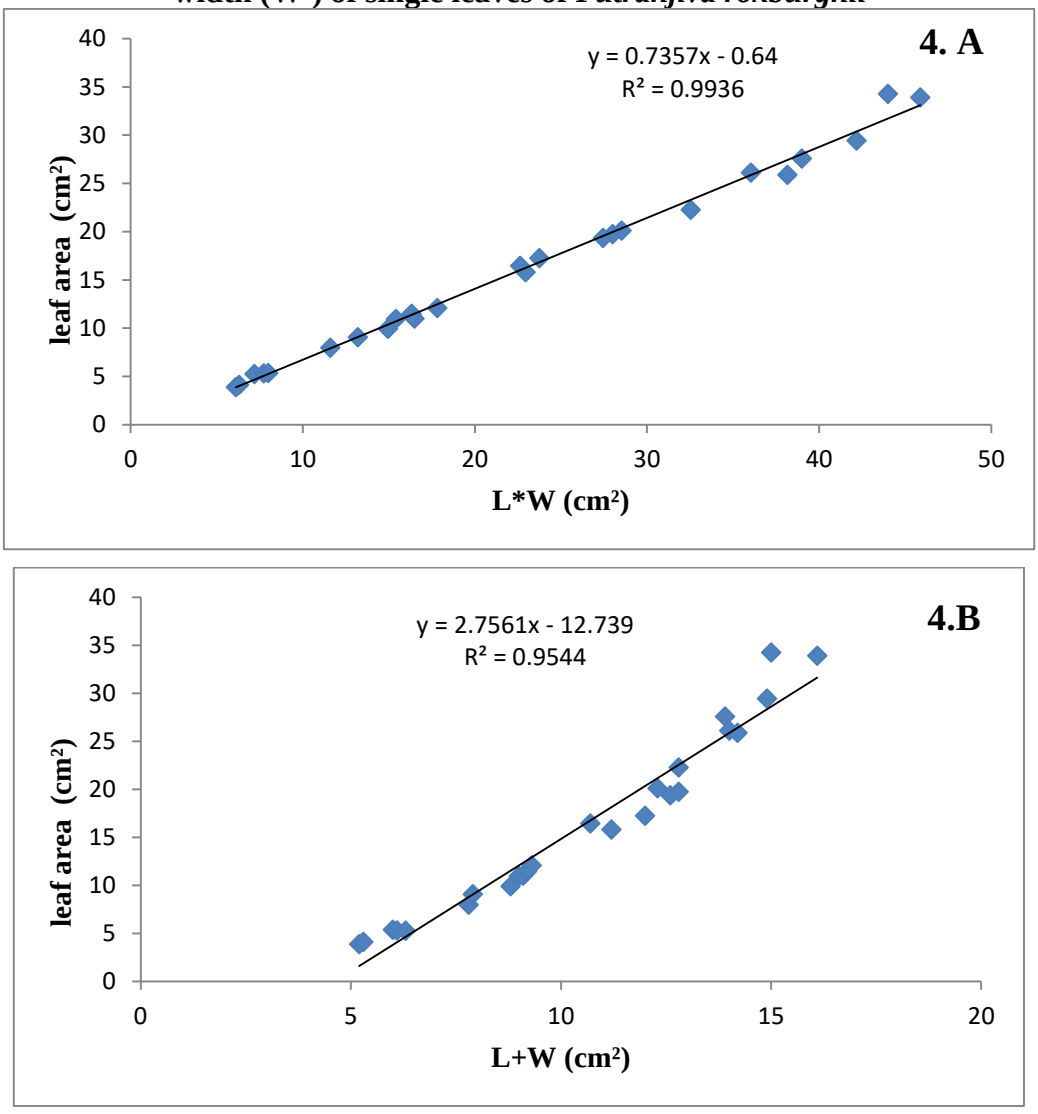


Figure 4: A -Relationship between leaf area (LA) and leaf length × leaf width and B- between (LA) and leaf length + leaf width of single leaves of *Putranjiva roxburghii*

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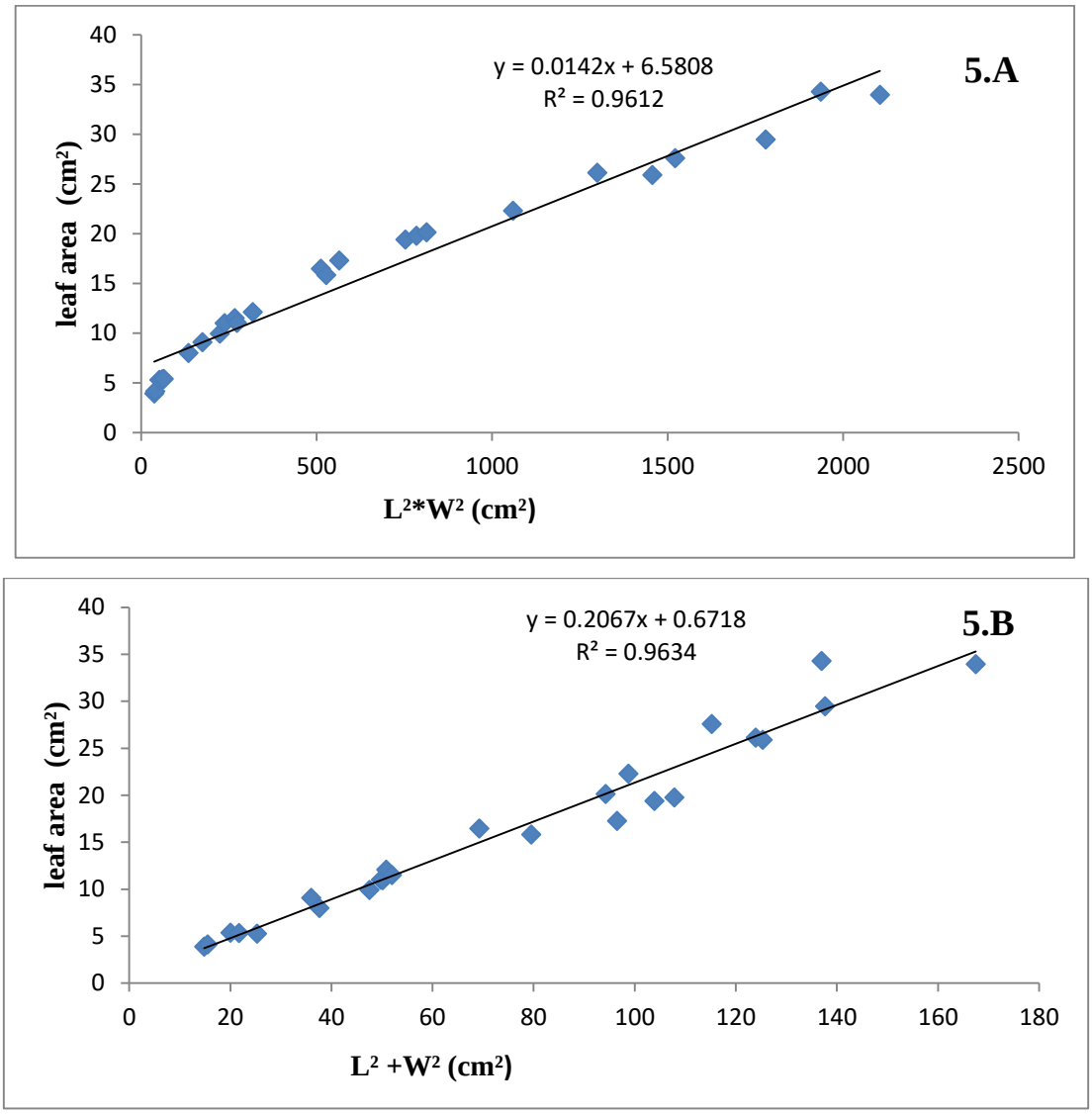
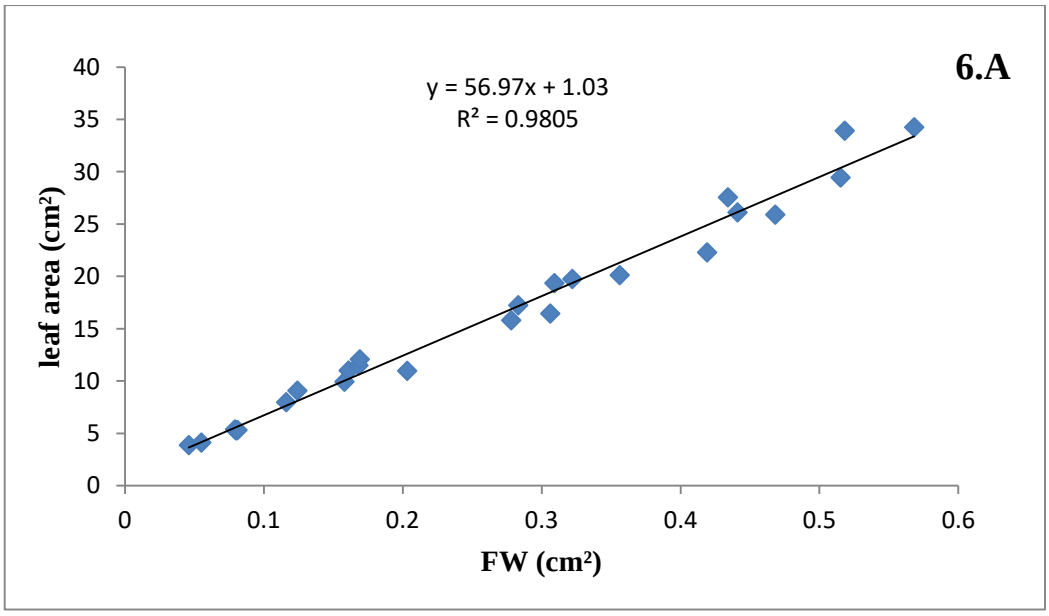


Figure 5: A -Relationship between leaf area (LA) and leaf length² + leaf width² and B- between (LA) and leaf length² × leaf width² of single leaves of *Putranjiva roxburghii*



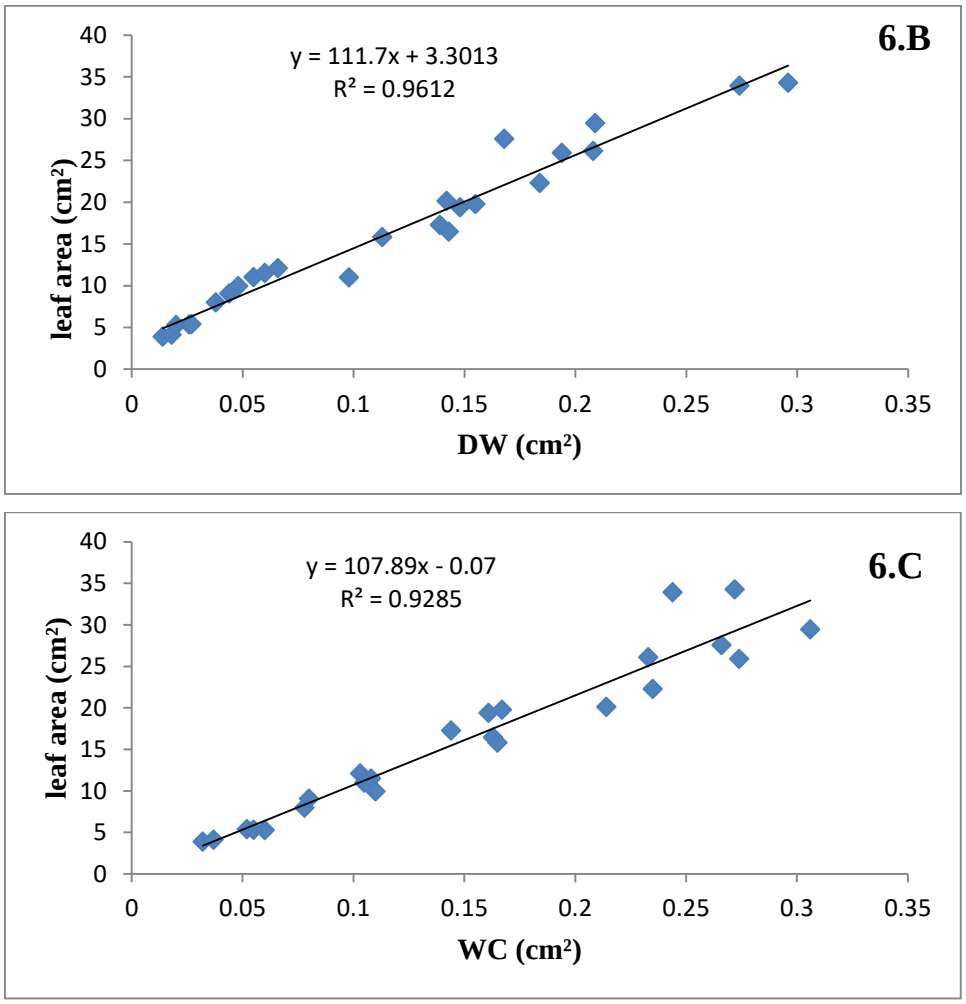


Figure 6: A -Relationship between leaf area (LA) and leaf fresh weight and B- between (LA) and B-leaf dry weight and C-leaf water content of single leaves of *Putranjiva roxburghii*

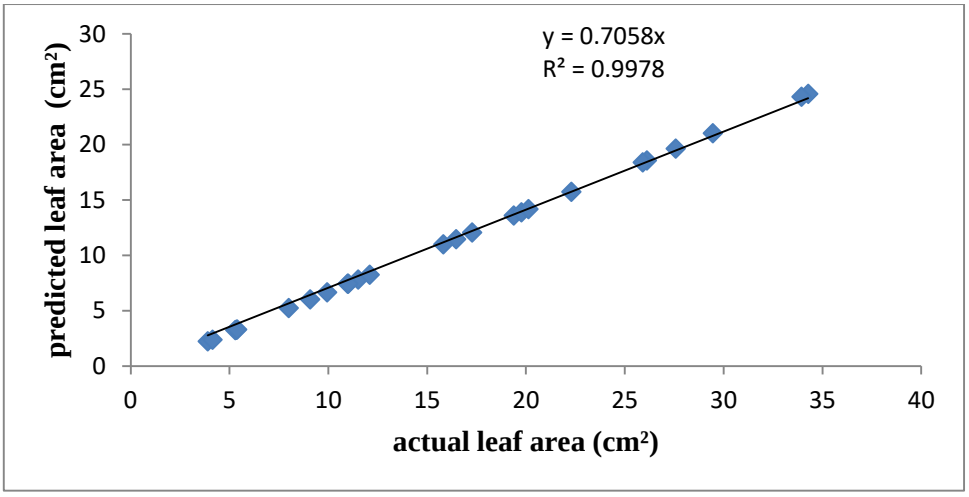


Figure 7: Comparison of actual and predicted leaf area in *Putranjiva roxburghii* (n=25)

4. CONCLUSION:

Result of the current study showed that area of Putranjiva leaves is well correlated to its length (LA = a + bL×W), the addition of its length² and width² (LA = a + b ($L^2 + W^2$), and its fresh weight (LA = a + bFW) with high R^2 values 0.9936, 0.9805 and 0.9634 respectively. The results from this study indicate that leaf area determination of Putranjiva could be estimated from the relationship with leaf length*width using linear equation Y = -0.64+0.7357L*W(R^2=0.9936) .This model would allow researchers to make non-destructive measurements and repeated measurements on the same leaves. This method showed high correlation in estimation of leaf area and can

accurately estimate the leaf area of individual leaves without the use of any expensive instrument. The leaf area of Putranjiva could also be estimated from the relationship with fresh weight using linear equation Y = 1.03+56.97 FW. From this study we can concluded that linear model no.5 (LA=a + bL*W) is the best linear model for the leaf area measurements for ***Putranjiva roxburghii*** with high r^2 value.(Table 2) and **the development of linear models for *Putranjiva roxburghii* is firstly reported in Gujarat during this study.**

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