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A Linear Model for Leaf Area Measurement of medicinally important plant: *Morinda citrifolia L.*

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Abstract: Leaf area is an important parameter in plant modelling studies, to develop physiological model in plant breeding .Leaf area is one of the vital growth factor for plants especially in medicinal plants and the limitation for calculating leaf area is the lack of accurate model. The plant *Morinda citrifolia* L. (Medicinal plant) was selected and by using counting grid method, the linear model was developed for the leaves of this plant. From the lower, middle and upper parts of the plant, different sizes of Leaf samples were randomly selected. 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 calculated and compared with actual leaf area measured by graph tracing method, to test their precision and reliability using Y = a + bX model. Between actual leaf area and the corresponding leaf length, width, L^2, W^2, product of these dimensions like leaf fresh and dry weights, there was a highly significant correlation (r=0.9225 to 0.9970). The product of the dimensions obtained from the regression analyses of LA versus FW, DW, L, W, L^2, W^2 revealed several models that could be used for estimating the area of individual leaf of plant. Among the models, the most accurate one was based on length and width dimension (LA = a + bL*W) r = 0.9970, R^2 = 0.9942. Actual leaf area of 25 leaf samples which was obtained by the graph tracing method was compared with leaf area estimated by the model for its validation. The leaf area estimated by the models strongly relates with the measured value of leaf area as evident from high value of R^2 (0.9998) for Morinda. The validation of the models indicate that model (LA = a + bL*W) was accurate and also reliable to determine the leaf area of the plant and hence it would be very useful for field workers who deals with large samples. **Development of the linear models for *Morinda citrifolia* L. is reported first in Gujarat during this study.**

Key Words: Medicinal plants, *Morinda citrifolia* L., Leaf area measurement, count grid method.

1. INTRODUCTION :

Morinda citrifolia L. is the small tropical evergreen tree of Rubiaceae family. It grows upto a maximum height of 10 m. It is native to south east Asia whose whole fruit, seeds ,juice, leaf, bark and root are used as sources of traditional medicines. Its cultivation has spread extensively to regions such as Mexico, Central and South America. Its economic value has grown significantly worldwide in recent years due to its health benefits. The largest market of *Morinda citrifolia* L. products are North America,. Europe, Japan, Mexico, Asia and Australia (A.Carrillo-lópez, E.M.Yahia 2011)[1]. *Morinda citrifolia* L. has various vernacular names which are “Indian mulberry”, “nuna” or “ach” on the Indian subcontinent “mengkudu” in Malaysia, “”nhau” in South East Asia, “painkiller bush” in the Caribbean or “cheese fruit” in Australia.(Morton,1992[2]; Nelson,2001[3]; Wang et al.[4],2002,Cardon, 2003[5]). Morinda citrifolia L. has several phytochemicals in it, of which around 160 compounds have been reported. The phytochemicals are mainly organic acids, phenolic compounds and alkaloids.(Wang and Su,2001)[6]. Morinda citrifolia L. is essential item in the diet of Burmese and Australian cultures. It has distinctive white, elongated , tubular flower, elliptical bright green large leaves, straight trunk, syncarpous fruits which has dark brown coloured seeds. (Nelson, 2005)[7]. Leaf area is an important variable for most ecophysiological studies in terrestrial ecosystem concerning light interception, evapotranspiration, photosynthetic efficiency, fertilizers and irrigation response & growth of the plant. (Blanco and Folegatti, 2005)[8]. Economic and precise estimate of leaf surface area has been a concern to plant scientists for a long time. There are different methodologies for the calculation of leaf area measurement which are as follows, counting grid squares, length, and width regression equation using planimeter instruction, paper weight model and digital image processing based method. Rapid, cheap and easy method to estimate leaf area can be of significant use to growers estimating leaf area from equations using simple measurement of leaf dimension is an inexpensive , rapid and non-

destructive alternative for accurately assessing leaf area (Tsialtas *et al* ., 2005)[9]. As a non-destructive method for the leaf area measurement linear models are developed for the plant species. So the establishment of mathematical and especially linear relationship between leaf area and one of more growth parameters of the leaf is an advantageous way to determine leaf area under field conditions and can be extended for follow up applications like preparation of herbal drugs (Kumbhani *et al.*,2017)[10]. As linear models for estimating the leaf area of *Morinda citrifolia* L. have not yet been established in Gujarat. With the objective of developing linear model for leaf area measurement of this plant, this study was undertaken by using the best matching regression equation of intact leaves of *Morinda citrifolia L.*and functions between plant LA and plant vegetative characteristics.

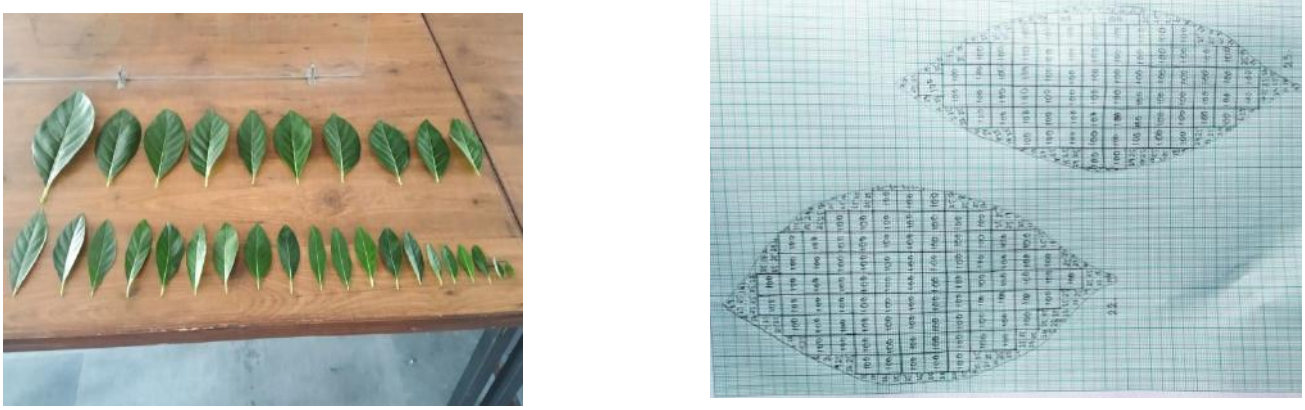
2. MATERIALS AND METHOD :

2.1 Collection of plant materials -

The leaves of Morinda *citrifolia L.* were collected from the botanical garden of M.G Science Institute, Ahmedabad, Gujarat. Leaves were collected and separated according to their size, smaller to larger.

2.2 Measurements of growth parameters

Taking into consideration the fresh weight, dry weight, water content, length, width and leaf area growth was measured. Freshly harvested smaller to larger sizes of leaves were taken for the measurement of fresh weight and dry weight. To obtain the data on fresh weight and dry weight the leaves collected were sundried to a constant weight for 6 -7 days. From the difference obtained between fresh and dry weight (gm), water content at each stage was determined. The length (cm), width (cm) and area (cm^2) of individual leaves were determined. Leaf length (L) and width (W) were measured with a simple ruler. By using counting grid square method according to Stewart and Dwyer,1999 [11] leaf area of 25 leaves was measured with some modifications. To identify appropriate functions for use in models estimating leaf area of the plant 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 ($L+W$, $L\times W$, L/W, $L^2\times W^2$, $L^2+ W^2$) and also with dry weight, fresh weight and water contents. Also the values of the coefficients (b) and constants (a) were reported. By fitting the equation the estimated leaf area was determined and based on the combination of the highest coefficient of determination (R^2) and correlation coefficient (r) the final model was selected.



A. **B.**

Fig.1 (A.) Leaves of *Morinda citrifolia L. and (B.) Counting grid square*

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 (*Morinda citrifolia L.)*

From *Morinda citrifolia L.*, 25 leaves were taken for the area measurement by using counting grid square method in which leaf area showed range between 3.09 to 238.3 cm^2. Minimum leaf area was observed 3.09 cm^2/ leaf whereas; maximum leaf area was 238.3 cm^2/leaf. Data on DW ranged between 0.01 to 1.355 mg/leaf, WC ranged between 0.065 to 6.515 mg/leaf and FW ranged between 0.075 to 7.87 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(3.1 cm ≤ length ≤ 28.1 cm, 1.5 cm ≤ width ≤ 13.7 cm, 9.61 cm^2 ≤ $length^2$ ≤ 789.61, 2.25 cm^2 ≤ $width^2$ ≤ 187.69, 4.6 cm ≤ length + width ≤ 41.8, 4.65 cm^2 ≤ length × width ≤ 384.97 cm^2, 11.86 cm^2 ≤ $length^2$ + $width^2$ ≤ 977.3, 21.62 cm^2 ≤ $length^2$ × $width^2$ ≤ 148202)in leaves which were used for the mathematical generation of models of leaf area estimation(Table.3).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=0.9225 to 0.9970) (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 = 0.9944,0.9936 and 0.9926 respectively).The obtained data was plotted against respective leaf area showed linear relationship.

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 ***Morinda citrifolia* L**. All models produced a coefficient of determination (R^2) equal to or greater than 0.9225. From the result of this study, models 1,2,3,6,7,8 are less acceptable for estimating leaf area of noni because of their lower coefficient of determination (R^2=0.8838,0.9575,0.9605,0.9293,0.851,and 0.9789 respectively) while model 4,5,9,10 and 11 are more acceptable for estimating leaf area of ***Morinda citrifolia* L**. because of their higher coefficient of determination (R^2= 0.9821,0.9942,0.9889,0.9873 and 0.9853 respectively) (Table.2)

3.1 Model validation for *Morinda citrifolia* L.

To validate the best model, different size of 25 leaves of noni 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 analyses 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 (cm^2), 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 (0.9998) (Figure 6). The validation of the model indicates that leaf area of *Morinda citrifolia* L. could be measured rapidly and accurately by using this linear developed model.

Table 1. Descriptive statistic of the leaf parameters measured during the experiment of *Morinda citrifolia* L.

	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	3.09	3.1	1.5	9.61	2.25	4.6	4.65	21.62	11.86	0.075	0.01	0.065
Max	238.3	28.1	13.7	789.61	187.69	41.8	384.97	148202	977.3	7.87	1.355	6.515
Mean	63.19	13.88	5.83	228.54	44.41	19.71	99..35	17988.63	272.95	1.75	0.32	1.43
SD	57.91	6.11	3.29	183.79	46.50	9.30	91.95	31349.43	228.63	1.84	0.34	1.50
Var	3353.84	37.38	10.83	33781.8	2162.53	86.56	8455.48	982786777	52274.4	3.40	0.11	2.26

Table 2.Fitted coefficient (b), constant (a), correlation coefficient (r) and coefficients of determination (r^2) values of the models used to estimate *Morinda citrifolia* L. 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	-60.398	8.9046	0.8838	0.9401
2	LA = a + bW	-37.211	17.217	0.9575	0.9785

3	$LA = a + bL^2$	-7.3761	0.3088	0.9605	0.9800
4	$LA = a + bW^2$	8.3868	1.2341	0.9821	0.991
5	**LA = a + bLW**	0.8072	0.628	0.9942	0.9970
6	LA = a + b (L + W)	-55.08	6.0003	0.9293	0.9639
7	$LA = a + b (L^2 + W^2)$	-5.2085	0.2506	0.9789	0.9894
8	$LA = a + bL^2W^2$	32.543	0.0017	0.851	0.9225
9	LA = a + bFW	8.5849	31.188	0.9889	0.9944
10	LA = a + bDW	9.5771	167.25	0.9873	0.9936
11	LA = a + bWC	8.5814	38.181	0.9853	0.9926

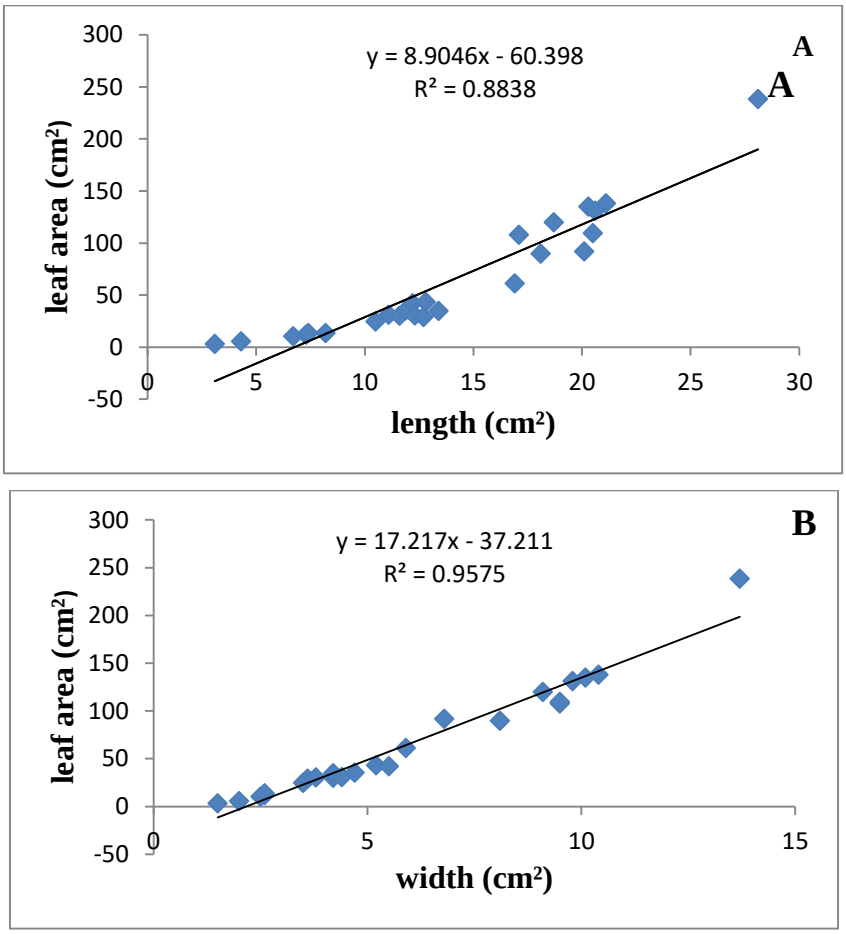
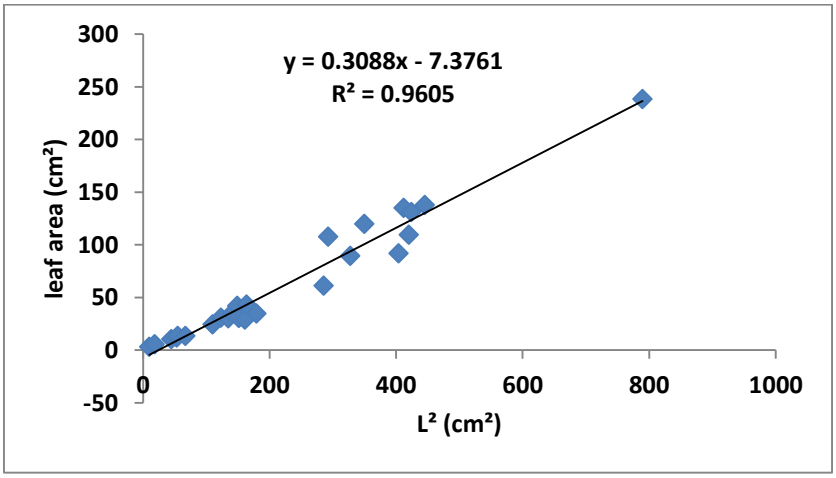


Figure 1. A-Relationship between leaf area (LA) and leaf length(L) and B- between leaf area(LA) and leaf width (W) of single leaves of *Morinda citrifolia* L.



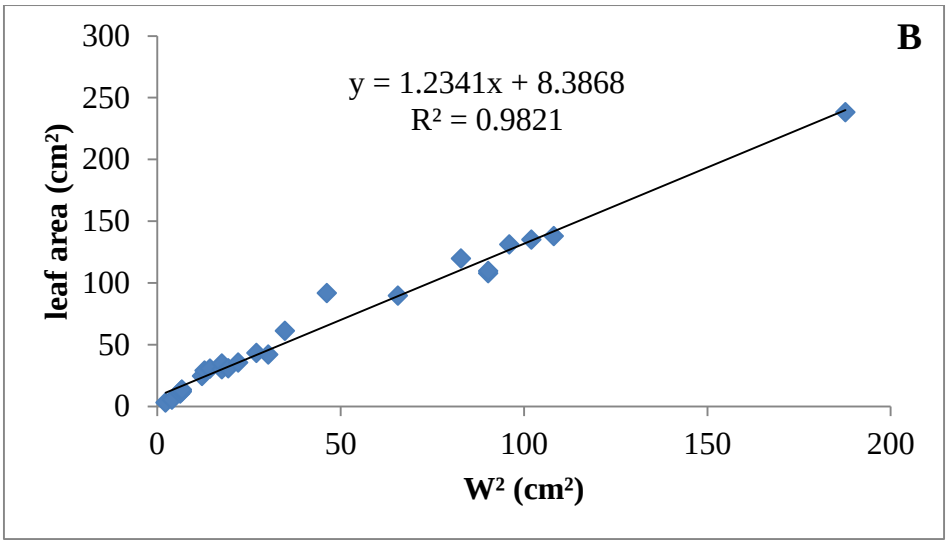
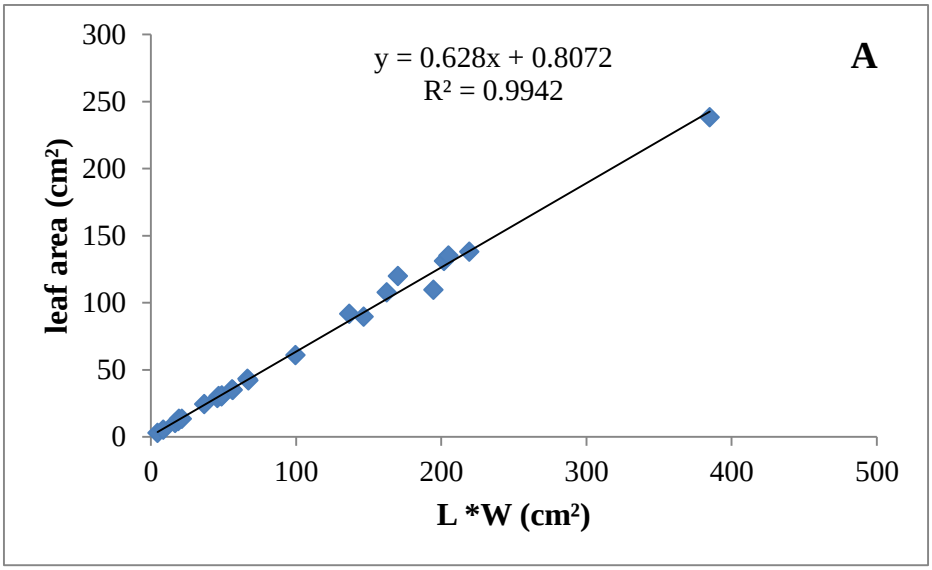


Figure 2. A - Relationship between leaf area (LA) and leaf length (L^2) and B- between (LA) and leaf width (W^2) of single leaves of *Morinda citrifolia* L.



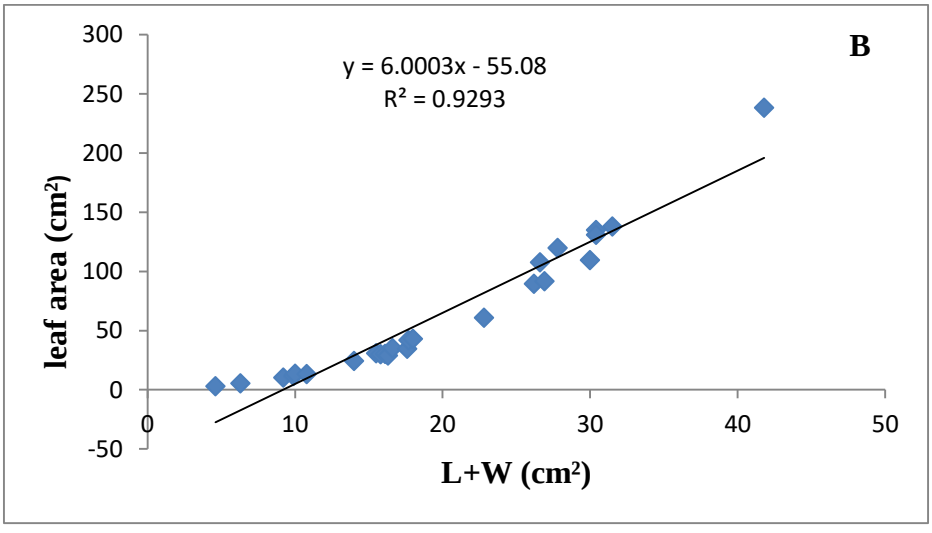


Figure 3. A -Relationship between leaf area (LA) and leaf length × leaf width and B- between (LA) and leaf length + leaf width of single leaves of *Morinda citrifolia* L.

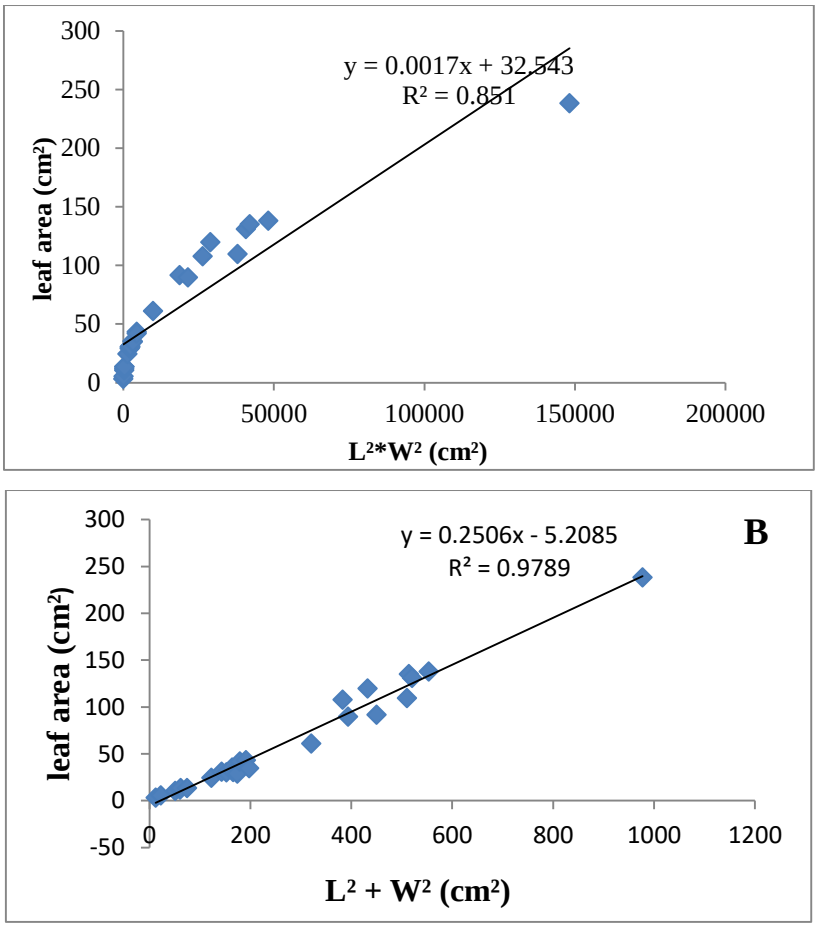
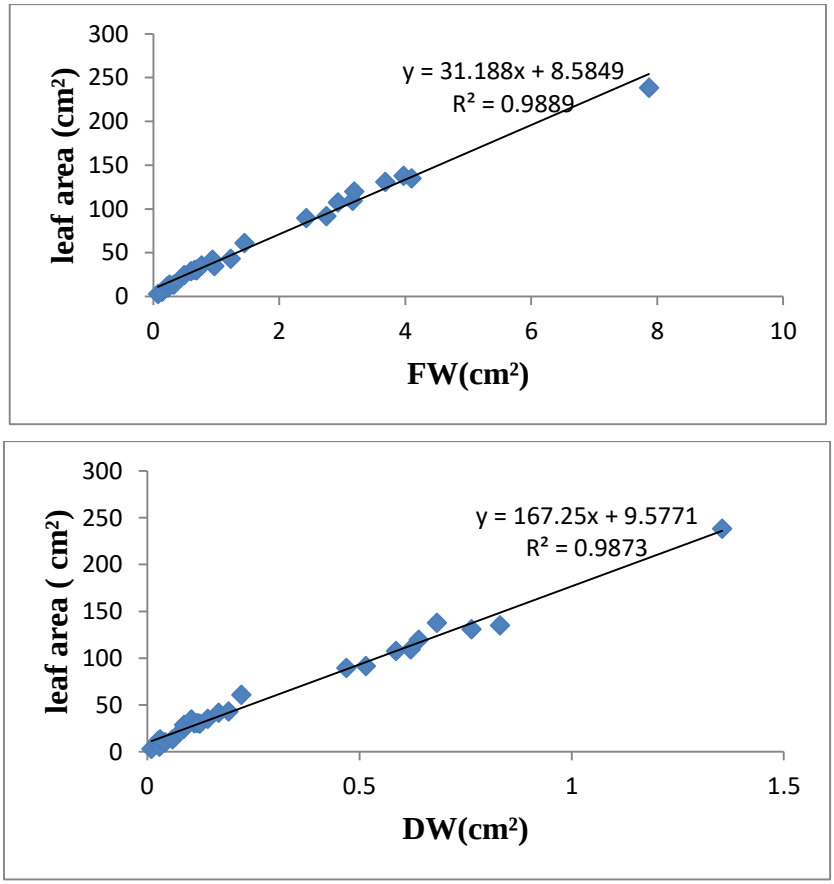


Figure 4. A -Relationship between leaf area (LA) and leaf length² + leaf width² and B- between (LA) and leaf length² × leaf width² of single leaves of *Morinda citrifolia* L.



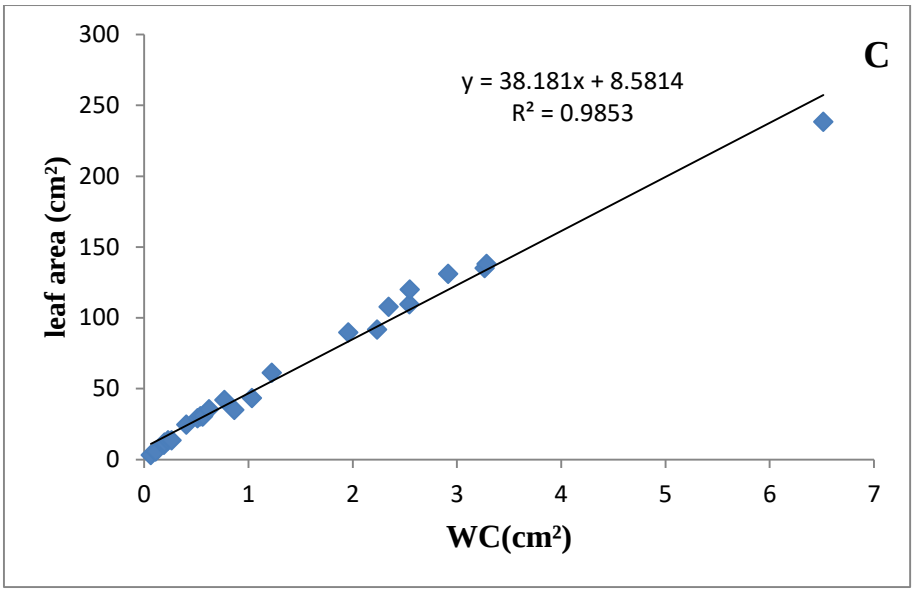


Figure 5. A -Relationship between leaf area (LA) and leaf fresh weight and B- between (LA) and B-leaf dry weight and C-leaf water content of single leaves of *Morinda citrifolia* L.

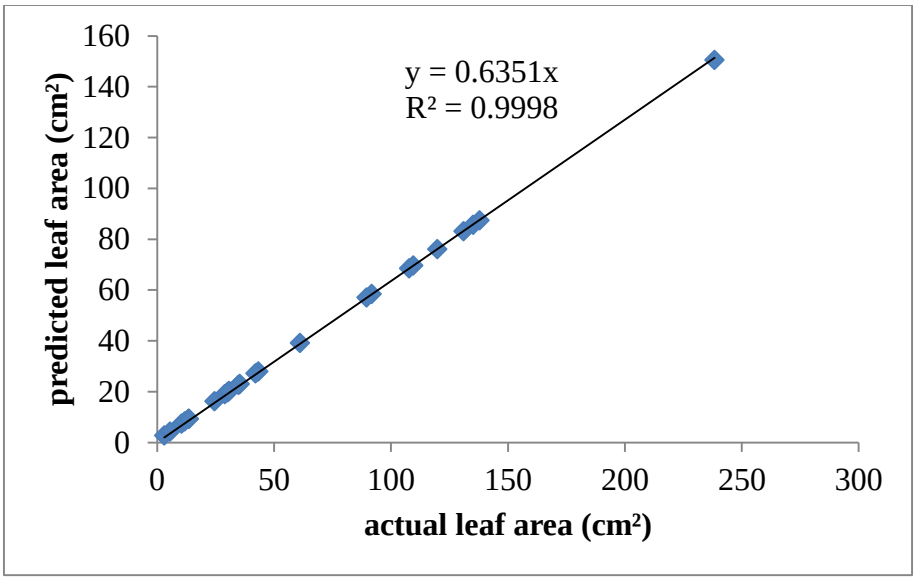


Figure 6. Comparison of actual and predicted leaf area in *Morinda citrifolia* L.(n=25)

4. CONCLUSION :

From this study the result indicate that leaf area determination of Morinda could be estimated from the relationship with leaf length*width using linear equation is Y= 0.8072+0.628L*W (R^2=0.9942). Researchers would be able to make non-destructive measurements and repeated measurements on the same leaves using this model. Without the use of any expensive instrument this method showed high correlation in estimation of leaf area and also can accurately estimate the leaf area of individual leaves. The leaf area of Morinda could also be estimated from the relationship with fresh weight using linear equation Y=8.5849+31.188FW, dry weight Y=9.5771+ 167.25DW.So we can conclude that linear model no.5 (LA=a + bL*W) is the best linear model for the leaf area measurements for ***Morinda citrifolia* L.** with high r^2 value(Table 2) from this study. **During this study the development of linear models for *Morinda citrifolia* L. is reported first in Gujarat .**

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