Hindawi Publishing Corporation
Journal of Botany
Volume 2011, Article ID 658240, 6 pages
doi:10.1155/2011/658240

Research Article

A Simple, Cost-Effective Method for Leaf Area Estimation

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Received 13 July 2011; Revised 25 August 2011; Accepted 1 September 2011

Academic Editor: Kang Chong

Easy, accurate, inexpensive, and nondestructive methods to determine individual leaf area of plants are a useful tool in physiological and agronomic studies. This paper introduces a cost-effective alternative (called here millimeter graph paper method) for standard electronic leaf area meter, using a millimeter graph paper. Investigations were carried out during August–October, 2009-2010, on 33 species, in the Botanical garden of the Banaras Hindu University at Varanasi, India. Estimates of leaf area were obtained by the equation, leaf area (cm^2) = x/y, where x is the weight (g) of the area covered by the leaf outline on a millimeter graph paper, and y is the weight of one cm^2 of the same graph paper. These estimates were then compared with destructive measurements obtained through a leaf area meter; the two sets of estimates were significantly and linearly related with each other, and hence the millimeter graph paper method can be used for estimating leaf area in lieu of leaf area meter. The important characteristics of this cost-efficient technique are its easiness and suitability for precise, non-destructive estimates. This model can estimate accurately the leaf area of plants in many experiments without the use of any expensive instruments.

1. Introduction

Leaf area is an important variable for most ecophysiological studies in terrestrial ecosystems concerning light interception, evapotranspiration, photosynthetic efficiency, fertilizers, and irrigation response and plant growth (Blanco and Folegatti [1]). The easy, economic, and precise estimate of leaf surface area has been a concern to plant scientists for a long time. Plant physiologists require leaf area measurements for studying primary production in plants (Sestak et al. [2]; Tieszen [3]; Bleasdale [4]). Ecologists use leaf area relations for elucidating competition among different plant species (Harper [5]). Leaf area estimate is valuable in studies of plant nutrition, plant competition, plant-soil-water relations, plant protection measures, respiration, light reflectance, and heat transfer in plants (Mohsenin [6]), and thus it is an important parameter in understanding photosynthesis, light interception, water and nutrient use, and crop growth and yield potential (Smart [7]; Williams [8]). Leaf area estimation is often costly, time-consuming, and destructive (Marshall [9]). Sestak et al. [2] provided an extensive 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. Well-known electronic meters can only be used if the plants have sparse and nonfragile leaves (Tieszen [3]; Bleadsdale [4]). A variety of computerized image analysis equipments and software are also available (Brodny et al. [10]). They measure quickly, accurately and nondestructively using a portable scanning planimeter (Daughtry [11]); however, the method is suitable only for small plants with few leaves (Nyakwende et al. [12]) and is expensive (Bignami and Rossini [13]). Several combinations of measurements and models relating length and width to area have been developed for several fruit trees, such as grape (Montero et al. [14]; Williams and Martinson [15]), avocado (Uzun and Celik [16]), pistachio (Ranjbar and Damme [17]), Cherry (H. Demirsoy and L. Demirsoy [18]), peach (Demirsoy et al. [19]), and Chestnut (Serdar and Demirsoy [20]). Some studies also use petiole length (Manivel and Weaver [21]) and leaf weight (Sepulveda and Kliewer [22]; Montero et al. [14]) for area measurement. The most common approach is to develop ratios and regression estimators by using easily measured leaf parameters such as length and width (Kvet and Marshall [23]). Lu et al. [24] proposed that the simple and linear relationships between leaf area and leaf dimensions (length, width) could be useful for nondestructive estimation of leaf area. Estimating leaf area from equations using leaf dimensions is an inexpensive, rapid, and nondestructive alternative for accurately assessing leaf area. Nondestructive models for leaf area determination have been established for many species such as maize (Stewart and Dwyer [25]), bean (Bhatt and Chanda [26]), taro (Lu et al. [24]), white clover (Gamper [27]), sugar beet (Tsialtas and Maslaris [28, 29]), sunflower (Kvet and Marshall [23], Rouphael et al. [30]), radish (Salerno et al. [31]), zucchini (Rouphael et al. [32]), strawberry (Demirsoy et al. [33]), grapevines (Manivel and Weaver [21]; Montero et al. [14], Williams and Martinson [15]), kiwi (Mendoza-de Gyves et al. [34]), chestnut (Serdar and Demirsoy [20]), hazelnut (Cristofori et al. [35]), eggplant (Rivera et al. [36]), faba bean (Peksen [37]), stevia (Ramesh et al. [38]), persimmon (Cristofori et al. [39]), medlar (Mendoza-de Gyves et al. [40]), small fruits (Fallovo et al. [41]), euphorbia (Fascella et al. [42]), saffron (Kumar [43]), ginger (Kandiannan et al. [44]), roses (Rouphael et al. [45]), and watermelon (Rouphael et al. [46]).

However, leaves may have complex shapes making leaf area determination using ratios of leaf parameters difficult, time consuming, and subject to larger errors. Therefore, the aim of this study was to develop an equation for leaf area estimate which is insensitive to changes in leaf shape, and is cost-effective. In this paper, a millimeter graph paper method is described, and its reliability is tested using an electronic leaf area meter.

2. Materials and Methods

Thirty-two (twelve- to thirty-five-year old) tree species and one (six-year old) shrub species growing at the Botanical garden of the Banaras Hindu University, Varanasi (25°18′ N and 80°01′ E, at 126 m above sea level, mean annual rainfall 1100 mm), were selected for the study. Leaves were sampled from different levels of the canopy, ten each from the thirty-three species, during the full-foliage period (August–October) in 2009-2010. Each leaf was spread over millimeter graph paper, and the outline of leaf was drawn. The leaf area of each leaf was measured using a leaf area meter (SYSTRONICS, Leaf Area Meter-211) having a sensor and read-out unit. Using the paper knife, the area of the millimeter graph paper covered by the outline was cut and weighed on an electronic balance. One cm^2 of the same millimeter graph paper was also cut and weighed. Leaves of some species were sampled more than once.

The following equation was used to calculate the leaf area nondestructively:

Leaf area (cm^2) = x/y, where x is the weight of the graph paper covered by the leaf outline (g) and y is the weight (g), of the cm^2 area of the graph paper. In addition, areas of ten leaves each from five species were measured using the leaf area meter while still attached to the plants. Outline of these attached leaf samples were also drawn on the millimeter graph paper. The area of the graph paper covered by the outline was cut and weighed. A one cm^2 of the millimeter graph paper was also cut and weighed. There were six hundred forty detached leaf samples (of thirty-three plant species), and fifty attached leaf samples (five plant species). Size of the leaves varied from 3.20 to 285.06 cm.

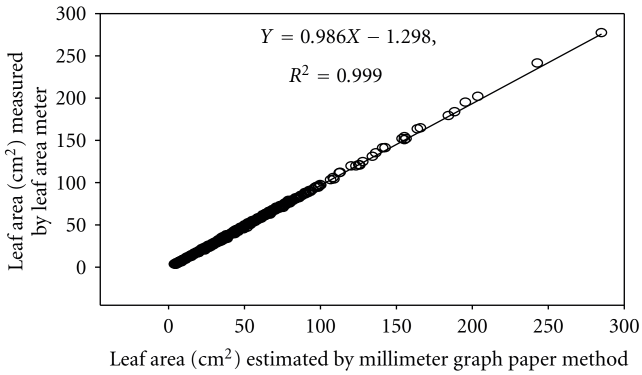

Figure 1: Relationship between leaf area of detached leaves measured by leaf area meter and that estimated by millimeter graph paper method across thirty-three plant species ($n = 640$).

The two sets of estimates (leaf area meter and millimeter graph paper) were related according to $y = a + bx$, where y is the leaf area estimated by leaf area meter and x is the leaf area estimated by millimeter graph paper. The independent variable here was the leaf area estimated by millimeter graph paper, and dependent variable was leaf area estimated by leaf area meter (SYSTRONICS, Leaf Area Meter-211). The regression equations were calculated by using Sigmaplot (ver.11).

3. Results and Discussion

Relationships between leaf area of detached leaves estimated by leaf area meter (dependent variable) and that estimated by millimeter graph paper method (independent variable) for thirty-three plant species as given in Table 1 show that the two sets of estimates are strongly related with each other for each of the thirty-three species and that the nondestructive estimates by millimeter graph paper method are as good as those obtained destructively by leaf area meter method (Figure 1). For individual species, the coefficient of determination between the two sets of estimates varied between 0.933 and 0.998 and collectively across the thirty-three, the R^2 was as high as 0.999. These relationships were also tested on attached leaf samples for five species (Table 2). Relationships were again linear and significant, (R^2 = 0.996 to 0.998, Figure 2).

Easily measured leaf parameters such as length and width, and their combinations have been used for nondestructive leaf area estimation, though the accuracy of the predictions is dependent on the variation of the leaf shape due to differential genotypes (Cristofori et al. [35], Cristofori et al. [39], Zhang and Liu [47]). The ratio of length to width is highly variable among the species due to complexity in the leaf shapes. On the other hand, the method using leaf

TABLE 1: Regression equations and coefficients of determination (R^2) between leaf area measured by leaf area meter (y, cm^2) and that estimated by millimeter graph paper method (x, cm^2) for thirty-three plant species, ($n = 10$ for each species). Observations were made on detached leaves.

S. no.	Species	Month of sampling	Regression equation	R^2
1	*Albizia lebbeck*	September	$Y = 0.742X + 0.741$	0.933
	Albizia lebbeck	September	$Y = 0.785X + 0.478$	0.859
	Albizia lebbeck	October	$Y = 1.093X - 1.685$	0.986
2	*Anacardium occidentale*	August	$Y = 0.986X - 1.159$	0.995
	Anacardium occidentale	September	$Y = 1.000X - 2.147$	0.991
3	*Anthocephalus cadamba*	September	$Y = 0.986X - 1.495$	0.997
4	*Artocarpus heterophyllus*	October	$Y = 0.984X - 0.947$	0.998
	Artocarpus heterophyllus	October	$Y = 0.972X - 0.894$	0.993
5	*Artocarpus lakoocha*	August	$Y = 1.085X - 2.753$	0.977
6	*Bombax ceiba*	August	$Y = 0.955X - 0.649$	0.993
	Bombax ceiba	October	$Y = 0.961X - 0.736$	0.996
7	*Buchanania lanzan*	August	$Y = 0.975X - 0.012$	0.998
8	*Butea monosperma*	August	$Y = 0.836X - 0.632$	0.953
	Butea monosperma	October	$Y = 0.985X - 0.952$	0.998
9	*Cassia fistula*	August	$Y = 0.942X - 0.321$	0.992
	Cassia fistula	September	$Y = 0.988X - 1.510$	0.997
10	*Crescentia cujete*	September	$Y = 0.976X - 0.426$	0.998
	Crescentia cujete	August	$Y = 0.983X - 1.458$	0.995
11	*Diospyros melanoxylon*	October	$Y = 0.973X - 0.954$	0.997
12	*Eucalyptus globulus*	September	$Y = 0.948X - 0.330$	0.991
13	*Ficus benghalensis*	September	$Y = 0.971X - 0.763$	0.997
14	*Ficus carica*	August	$Y = 0.986X - 1.170$	0.994
15	*Ficus religiosa*	September	$Y = 1.000X - 1.879$	0.997
	Ficus religiosa	October	$Y = 0.991X - 2.296$	0.997
	Ficus religiosa	October	$Y = 0.995X - 1.863$	0.998
16	*Hibiscus rosa-sinensis*	October	$Y = 0.970X - 0.813$	0.995
17	*Holoptelea integrifolia*	August	$Y = 0.982X - 1.367$	0.998
	Holoptelea integrifolia	October	$Y = 0.962X - 0.478$	0.998
18	*Litchi chinensis*	August	$Y = 0.989X - 2.009$	0.995
	Litchi chinensis	August	$Y = 0.970X - 0.816$	0.996
	Litchi chinensis	October	$Y = 0.964X - 0.862$	0.993
19	*Madhuca indica*	August	$Y = 0.991X - 1.925$	0.995
	Madhuca indica	October	$Y = 0.990X - 1.933$	0.998
20	*Mangifera indica*	August	$Y = 0.997X - 1.801$	0.998
	Mangifera indica	October	$Y = 0.983X - 1.241$	0.996
	Mangifera indica	October	$Y = 0.996X - 1.177$	0.998
21	*Melia azadirachta*	August	$Y = 1.007X - 1.338$	0.977
	Melia azadirachta	September	$Y = 1.023X - 1.122$	0.969
22	*Morus alba*	August	$Y = 0.973X - 0.735$	0.996
	Morus alba	September	$Y = 0.978X - 0.534$	0.998
23	*Polyalthia longifolia*	August	$Y = 0.966X - 0.644$	0.998
	Polyalthia longifolia	August	$Y = 0.967X - 0.887$	0.998
	Polyalthia longifolia	September	$Y = 1.014X - 2.366$	0.993
24	*Populus alba*	August	$Y = 0.956X - 0.708$	0.993
25	*Psidium guajava*	September	$Y = 0.969X - 0.600$	0.994
	Psidium guajava	September	$Y = 0.959X - 0.634$	0.997

Table 1: Continued.

S. no.	Species	Month of sampling	Regression equation	R^2
26	*Sapindus emarginatus*	August	$Y = 0.98\,X - 1.097$	0.997
	Sapindus emarginatus	September	$Y = 0.967X - 0.787$	0.989
	Sapindus emarginatus	September	$Y = 0.976X - 1.025$	0.997
	Sapindus emarginatus	September	$Y = 0.997X - 1.474$	0.998
	Sapindus emarginatus	August	$Y = 0.987X - 1.202$	0.992
27	*Shorea robusta*	August	$Y = 0.97\,X - 0.735$	0.997
	Shorea robusta	October	$Y = 0.997X - 2.629$	0.998
28	*Sporadic pinata*	August	$Y = 0.959X - 0.475$	0.995
	Sporadic pinata	October	$Y = 1.024X - 3.014$	0.996
29	*Sterculia colorata*	October	$Y = 1.011X - 3.651$	0.998
	Sterculia colorata	August	$Y = 1.002X - 2.912$	0.998
30	*Syzygium jambolanum*	September	$Y = 0.964X - 0.065$	0.996
	Syzygium jambolanum	September	$Y = 1.002X - 1.887$	0.997
	Syzygium jambolanum	October	$Y = 0.976X - 0.709$	0.998
31	*Tectona grandis*	September	$Y = 0.996X - 2.180$	0.998
32	*Terminalia chebula*	October	$Y = 0.979X - 0.767$	0.998
33	*Terminalia tomentosa*	August	$Y = 0.977X - 0.676$	0.997
	Terminalia tomentosa	October	$Y = 0.974X - 1.089$	0.992

All R^2 values are significant at $P < 0.0001$.

Table 2: Regression equations and coefficients of determination (R^2) between leaf area measured by leaf area meter (y, cm^2) and that estimated by millimeter graph paper method (x, cm^2) for five plant species, ($n = 10$ for each species). Observations were made on attached leaves.

S. no.	Species	Month of sampling	Regression equation	R^2
1	*Crescentia cujete*	August	$Y = 0.953X - 1.247$	0.998
2	*Hibiscus rosa-sinensis*	October	$Y = 0.984X - 2.436$	0.996
3	*Morus alba*	September	$Y = 0.979X - 1.580$	0.998
4	*Populus alba*	August	$Y = 0.967X - 1.537$	0.998
5	*Psidium guajava*	September	$Y = 1.030X - 2.483$	0.997

All R^2 values are significant at $P < 0.0001$.

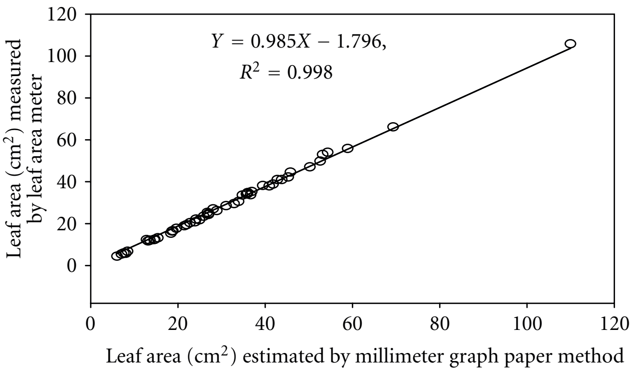

Figure 2: Relationship between leaf area of attached leaves measured by leaf area meter and that estimated by millimeter graph paper method across five plant species ($n = 50$).

outline on millimeter graph paper can be successfully used to estimate leaf area across variety of species. Some important factors which affect the accuracy of the millimeter graph paper method are the lack of proper spread of leaf over millimeter graph paper, absence of accurate drawing of leaf margins, lack of even cutting of the drawn outline, and lack of precision in weighing. The errors originating from the leaves not being perfectly flat, overlying leaflets, and similar factors are common to both the millimeter graph paper and leaf area meter. The millimeter graph paper method is faster and can be applied to attached leaves (nondestructive) and anywhere as in forest or agricultural field.

4. Conclusion

The millimeter graph paper method described in this paper was used to estimate individual leaf area of thirty-three woody species. The estimates had significant linear relationships with the estimates obtained by using sophisticated leaf area meter. The millimeter graph paper method can estimate precisely and in large quantities leaf area of plants in many experimental comparisons without the use of costly instruments.

Acknowledgment

The authors thank the Ministry of Environment and Forests, Government of India, for providing financial assistance.

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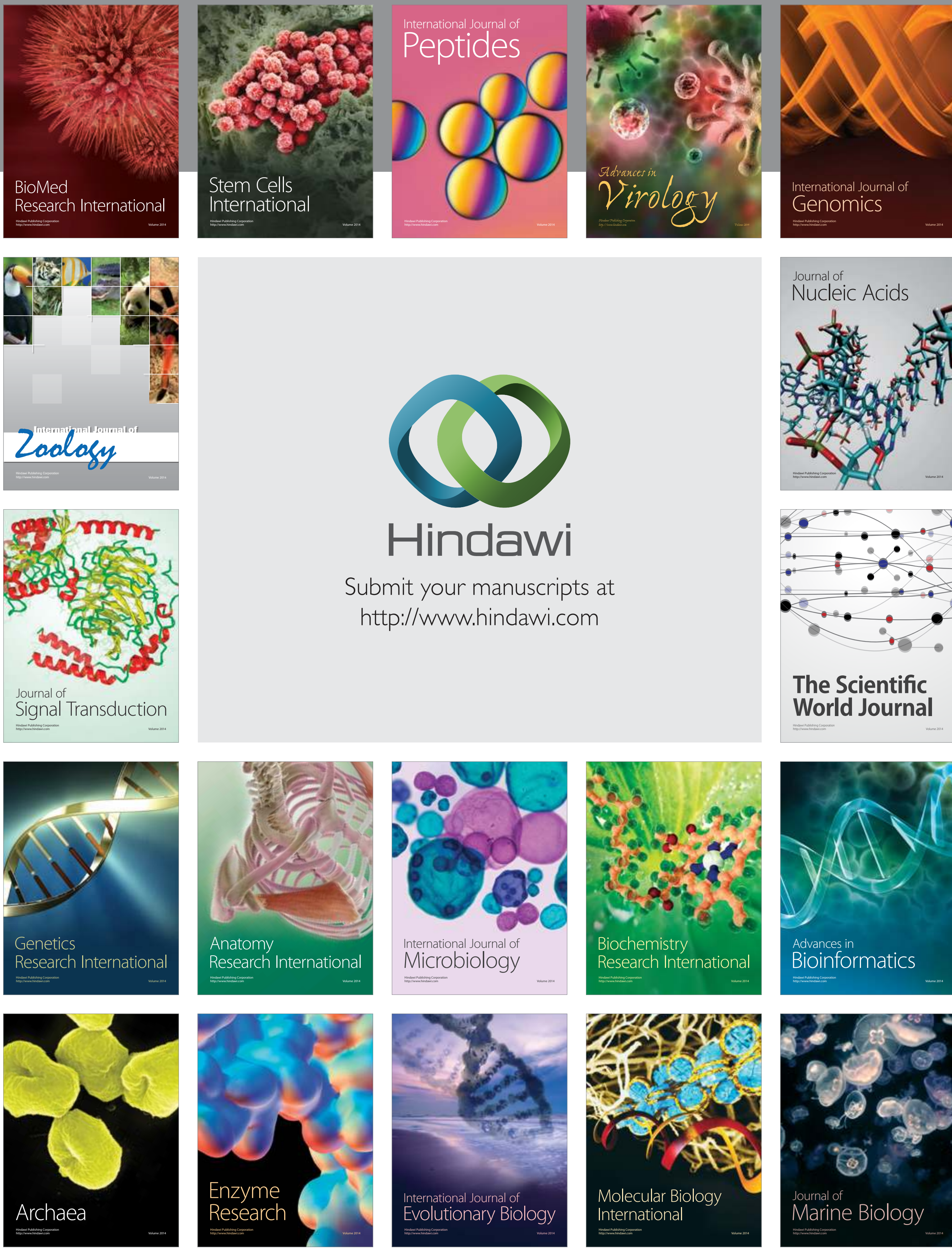
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