academicJournals

Vol. 7(20), pp. 1467-1474, 25 May, 2013
DOI: 10.5897/JMPR12.967
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Simulation and validation of leaf area prediction model for *Picrorhiza kurroa* – An endangered medicinal plant of Western Himalaya

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Accepted 16 April, 2013

The study was aimed at modeling of individual leaf area of *Picrorhiza kurroa* using linear measurements of leaf length (L) and maximum width (W). Leaves were collected from the greenhouse at different time intervals during 2009 and 2011. The actual leaf area (LA) and leaf dimensions were measured with a laser area meter. Different combinations of prediction equations were obtained from length (L), width (W), product of LW to build linear ($y = a + bx$), quadratic ($y = a+bx+cx^2$), exponential ($y = ae^{bx}$), logrithmatic ($y = a+bLnx$) and power models ($y = ax^b$) for different samples and pooled data compared with earlier models by graphical procedures and statistical criteria root mean square error (RMSE). A linear model having LW as the independent variables ($y = 0.333 + 0.603LW$) provided the most accurate estimate ($R^2 = 0.955$, RMSE = 0.573, coefficient of variation (CV) = 7.46%) of *P. kurroa* leaf area. Validation of the regression model having LW of leaves measured in two different experiments during September, 2011 showed that the correlation between measured and predicted values by the use of this equation was very high ($R^2 = 0.9053$), with low RMSE (0.39) and CV (5.44%).

Key words: *Picrorhiza kurroa,* leaf area model, non destructive, validation.

INTRODUCTION

Picrorhiza kurroa Royal ex Benth (family Scrophulariaceae) is a perennial herb with stout creeping stolon. It is one of the important medicinal plants of Himalaya having hepatoprotective activities. The plant grows naturally from 2,800 to 5,000 meters above sea level in alpine region. Underground parts (roots and rhizomes) are used for extraction of picrosides, the medicinally important constituents of *P. kurroa* but Singh et al. (2011) and Kumar et al. (2012) have reported the presence of picrosides in leaf tissue. Its root and rhizomes are used to treat disorders of the liver and upper respiratory tract, dyspepsia, chronic diarrhoea and scorpion sting (Visen et al., 1998; Verma et al., 2009).

Accurate assessment of leaf area is essential for the evaluation of plant performance while studying physiological and agronomic experiments (Meier and Leuschner, 2008). Leaf area strongly influences growth and productivity; therefore estimating this parameter is a fundamental component of crop growth models (Lizaso et al., 2003). Several methods of leaf area measurement *viz*., tracing, blueprinting, photographing, and conventional planimeter are there but they required the leaf detachment from the plants. Thus, successive measurements of the same leaf sample are not possible. A costly instrument like leaser leaf area meter is also there which can measure the leaf area without detaching the leaf, but

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for smaller leaf surface it cannot be used. Portable scanning planimeter (Daughtry, 1990) can also be used to measure leaf area quickly, accurately, and non-destructively but it is only suitable for small plants with few leaves (Nyakwende et al., 1997).

Although several models have been used to estimate leaf area (Williams and Martinson, 2003; Lu et al., 2004; Rouphael et al., 2006; Hyojin and Park, 2009; Akbulut and Ozkan, 2009) but the most common approach for nondestructive estimation of leaf area uses regression equations based on leaf dimensions such as mid vein length and maximum width of the leaf. This method reduces the variability associated with destructive sampling procedures (Ne Smith, 1992). It is simple, saves time, non-destructive and appropriate for research involving multiple measurements of leaf area on the same plant during plant growth. Estimation of leaf area from mathematical models involving linear measurements of leaves is relatively accurate and non-destructive.

A mathematical model can be obtained by correlating the leaf length (L), width (W) or length × width (LW) to the actual leaf area (LA) of a sample of leaves using regression analysis. The non-destructive methods based on linear measurements are quicker and easier to be executed and present good precision and high accuracy as demonstrated for several medicinal plants (Odabas et al., 2005; Crak et al., 2005), stevia (Ramesh et al., 2007), ginger (Kandiannan et al., 2009), saffron (Kumar, 2009), clary sage (Kumar and Sharma, 2010), rose (Rouphael et al., 2010), oregano (Caliskan et al., 2010) and *Bergenia purpurascens* (Zhang and Liu, 2010) etc. These models are based on leaf L and W measurements, which are used individually or in combination in order to establish linear, quadratic, or exponential functions and the best-fitted curve to be chosen.

Although, several leaf area prediction models are available to estimate leaf area for numerous crops, but the information on the estimation of *P. kurroa* leaf area is still lacking and no such model is available throughout the world. The equations produced for different crops in literature revealed that the coefficients as well as type of equations are crop specific. Therefore, the aim of this work was to develop reliable non destructive leaf area estimation model for *P. kurroa* using linear measurements.

MATERIALS AND METHODS

Experimental site

P. kurroa plants of different accessions were grown in pots under greenhouse conditions at Chandpur farm of CSIR-Institute of Himalayan Bioresource Technology (Council of Scientific and Industrial Research), Palampur, India during October, 2008. Geographically, the experimental site is situated at 32° 6'N latitude and 76° 3'E longitude at an elevation of about 1325 m a. s. l. in North-western Himalayas, and has a mean annual temperature of 18°C. Rainy season accounts for about 65% of the total annual rainfall exceeding 2,500 mm, and is consequently associated with low sun shine hours. Standard crop management practices were followed and need based crop protection measures was resorted.

Calibration of the model

At maximum vegetative stage of the crop, 100 representative leaves from each were selected at random among three accessions of *P. kurroa viz.* IHBT-PK-3, IHBT-PK-18 and IHBT-PK-26, and thus total of 300 measurable leaves were detached carefully in the preliminary calibration experiment. Leaf area (LA), length (L), width (W), and length width ratio (L/W) of all leaves were measured with a laser area meter CI 203 CID, Inc., USA. The sampled leaf range for model simulation and its validation are shown in the Table 1. This work examined the relationship between area per leaf and length and width dimensions in an attempt to identify appropriate functions for use in models estimating leaf area of *P. kurroa*. The relationship between leaf area as a dependent variable and L, W, LW, L/W, $L^{0.5}$, L^2, $W^{0.5}$, W^2, L^2W^2 as independent variables was determined using regression analysis on data of individual and together in combination of all the stages during both the years.

Coefficients of determination (R^2) were calculated and the equation that presented the highest R^2 was used in the estimations. The linear (y = a + bx), quadratic ($y = a + bx + cx^2$), power ($y = ax^b$), exponential ($y = ae^{bx}$) and logrithmatic (y = a + bLnx) model equations and multiple regression analysis were developed with Microsoft excel 7.0 computer package programme separately, where 'y' is the measured leaf area (cm^2), 'a' is the intercept, 'b' is the slope, 'c' is the constant and 'x' is the independent variable. The estimated leaf area was determined by fitting the equation. To evaluate the accuracy of the forecasting and to compare the models, different measures including, R^2, RMSE and mean square error (MSE) were used (Waller, 2003). Predicted residual error sum of squares (PRESS), a statistics based on the leave-one-out technique proposed by Allen (1974), was also used to compare different models. The final model was selected based on the combination of the highest R^2 and the lowest RMSE.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (Sim.Yi - Obs.Yi)^2}{n}}$$

$MSE = RMSE^{1/2}$

Where 'n' is the number of observations, *'Sim Yi' and 'Obs Yi'* are the simulated and observed leaf area values of i^{th} observation. The RMSE tests the accuracy of the model which is defined as the extent to which predicted values approach a corresponding set of measured values. Beside this, coefficient of variation (CV) was also used to validate the models. CV was calculated from the following equation:

CV (%) = RMSE × 100 / x

Where 'x' is the mean observed values. Because using two measurements 'L' and 'W' (which was the best model for estimating leaf area) introduces potential problems of collinearity, this result in poor precision in the estimates of the corresponding regression coefficients. For detecting collinearity, the variance inflation factor (VIF) (Marquardt, 1970) and the tolerance values (T) (Gill, 1986) were calculated by the following formulae:

Table 1. Sample range for model development and validation

Accession	No. of leaves	Leaf length (cm)			Leaf width (cm)			Leaf area (cm^2)		
		Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean
2009										
IHBT-PK-3	100	6.87	1.72	4.05	3.30	1.08	1.71	7.62	1.66	3.98
IHBT-PK-18	100	8.40	1.97	5.28	3.23	0.53	2.25	14.97	0.88	7.72
IHBT-PK-26	100	9.67	1.55	6.61	4.00	0.11	2.71	18.27	2.65	11.48
2011										
IHBT-PK-3	100	10.45	1.10	5.57	3.61	0.11	1.95	13.91	0.12	2.39
IHBT-PK-18	100	13.52	2.50	7.39	3.85	1.41	2.43	15.12	1.46	7.37
IHBT-PK-26	100	11.27	2.20	6.70	4.06	1.35	2.41	15.58	1.13	5.98
September, 2011	150	12.27	2.12	6.87	4.60	1.26	2.40	21.87	1.25	7.22

Min = minimum, Max = maximum

$$VIF = \frac{1}{1 - R2}$$

$$T = \frac{1}{VIF}$$

Where '*R*' is the correlation coefficient. If the VIF value was higher than 10 or if T value was smaller than 0.10, then collinearity may have more than a trivial impact on the estimates of the parameters and consequently one of them should be excluded from the model (Cristofori et al., 2007; Fallovo et al., 2008).

Model validation

To validate the developed model, about 150 leaves of *P. kurroa* were taken from different experiment during September, 2011. Actual leaf area, leaf length and width were determined by the previously described procedures. Leaf area of individual leaves was predicted using the best model from the calibration experiment and was compared with the actual leaf area. The slope and intercept of the model were tested to see if they were significantly different from the slope and intercept of the 1:1 correspondence line (Dent and Blackie, 1979). Regression analyses were conducted.

RESULTS

Development of prediction equations

Correlation coefficients for the leaf area per leaf determined by leaf area meter and for dependent variables L, L^2, $L^{0.5}$, W, W^2, $W^{0.5,}$ LW and L^2W^2 were calculated during different growth stages. The R^2 defined as the ratio of the sum of the squares due to regression and total sum of squares had been considered. Regression model with highest R^2 value was considered as best prediction model. Based on R^2 value, it was found that the use of both length and breadth of the leaves best represented the actual leaf area of *P. kurroa*. In order to establish a more accurate LA prediction model, L^2 and W^2, L^2W^2, were used as suggested by other researchers (Salerno et al., 2005; Serdar and Demirsoy, 2006). As a preliminary step to model calibration, the degree of collinearity among 'L' and 'W' was analyzed. The VIF ranged from 1.0 to 1.9, and T values ranged from 0.65 to 1.00, depending on the growth stage. In all, VIF was < 10, and T was > 0.10, showing that the collinearity between 'L' and 'W' can be considered negligible (Gill, 1986) and these both variables be included in the model.

Relation between leaf length, width and leaf area

Leaf area prediction equations, when 'L' and 'W' considered together for leaf area estimation, are presented in (Table 2). Leaf area prediction equations considering product of leaf length and width as independent variable for leaf area estimation shows 87 to 98% association with actual leaf area at different stages of plant growth (Figure 1). There was a very close relationship between actual leaf area and predicted leaf area which suggests that it is highly reliable across a range of cultivars and is being open to being evaluated. RMSE ranged from 0.40 to 1.77 and CV from 7.32 to 29.56%.

For each group, the fitted models were ranked according to their R^2. The model with the highest R^2 most frequently across all groups was regarded as the best model. From the above, based on R^2 value it was found that the use of both length and width of the leaves best represented the actual leaf area. Relationship between length and width product and actual leaf area for different models are depicted in Figure 1. A linear model having LW as an independent variable showed to have a rather high R^2 and thus it could serve as an accurate LA prediction

Table 2. Regression models for the estimation of leaf area of *P. kurroa*.

Model	Accession	Constant			R^2	MSE	PRESS	RMSE	CV (%)
		a	b	c					
2009									
y=a+bx	IHBT-PK-3	0.809	0.456	-	0.846	0.18	17.66	0.420	10.54
	IHBT-PK-18	0.333	0.603	-	0.955	0.33	32.86	0.573	7.46
	IHBT-PK-26	1.673	0.545	-	0.847	1.03	102.71	1.013	8.83
	Combined-2009	-0.092	0.631	-	0.954	1.99	198.72	0.814	10.54
$y=a+bx+cx^2$	IHBT-PK-3	-0.173	0.725	-0.0168	0.858	0.16	16.31	0.404	10.13
	IHBT-PK-18	-0.168	0.695	-0.0037	0.957	0.32	31.62	0.562	7.32
	IHBT-PK-26	2.001	0.503	0.0012	0.847	1.03	102.56	1.013	8.82
	Combined-2009	-0.366	0.681	-0.0019	0.954	1.97	197.31	0.810	10.51
$y=ax^b$	IHBT-PK-18	0.694	0.962	-	0.979	0.34	33.50	0.570	7.53
2011									
y=a+bx	IHBT-PK-3	0.134	0.355	-	0.903	0.57	56.85	0.754	16.02
	IHBT-PK-18	1.043	0.343	-	0.844	1.52	151.94	1.230	16.71
	IHBT-PK-26	0.397	0.344	-	0.852	0.98	98.18	0.991	16.58
	Combined-2011	0.352	0.358	-	0.870	3.37	336.54	1.059	17.60
$y=a+bx+cx^2$	IHBT-PK-3	0.923	0.230	0.0040	0.909	0.53	53.36	0.730	15.52
	IHBT-PK-18	0.756	0.376	-0.0008	0.845	1.52	151.50	1.230	16.69
	IHBT-PK-26	0.270	0.360	-0.0004	0.852	3.12	312.11	1.767	29.56
	Combined-2011	0.320	0.362	-0.0001	0.870	3.37	336.52	1.059	17.60
$y=ax^b$	IHBT-PK-18	0.413	0.957	-	0.876	1.03	102.54	1.012	16.94
	Combined-2011	0.447	0.938	-	0.875	3.53	352.90	1.080	18.02

model for *P. kurroa* cultivars (Figure 1). Among all the models, quadratic model {y = - 0.168 + 0.695 (LW) + (-0.0037) × (LW^2)}, power model (y = 0.694 $LW^{0.962}$) and linear equation (y = 0.333 + 0.603 LW) provided lesser MSE, RMSE, CV and PRESS (predicted residual error sum of squares) and slightly higher R^2 as compared to other equations but linear equation (y = 0.333 + 0.603 LW) was chosen for estimation of leaf area of *P. kurroa* due to simplicity and easy calculations as there was not much difference between MSE, RMSE, CV and PRESS and R^2 of these equations.

Validation

Comparisons were made between measured versus calculated leaf area of leaves collected from different experiment by using equation (y = 0.333 + 0.603 LW). For validating the model, 150 represented leaf samples *P. kurroa* were taken during September, 2011 from different experiment. Their area was measured with leaf area meter and area was predicted by fitting the equation (y = 0.333 + 0.603 LW). Similarly, predicted and actual area was compared. The leaf area estimated by the model strongly agreed with the measured value of leaf area of the leaves as evident from higher value of R^2 (0.9053) and lower values of RMSE (0.39) and CV (5.44%). The linear regression for the relationship between measured and estimated values was not significantly different from the 1:1 line (Figure 2). It suggested that linear model can well be used to predict *P. kurroa* leaf area.

DISCUSSION

A low MSE, RMSE, CV and PRESS showed that a calculated LA is close to the measured one and thus RMSE and CV should be the main criterion for selecting LA depiction model when a precise estimation of the LA is necessary. The combination of *L* and *W* shared higher precision than as individual, either L or W (Table 1). R^2 was reported to be a good measure of predictive ability of a model. All regression was significant, and the entire

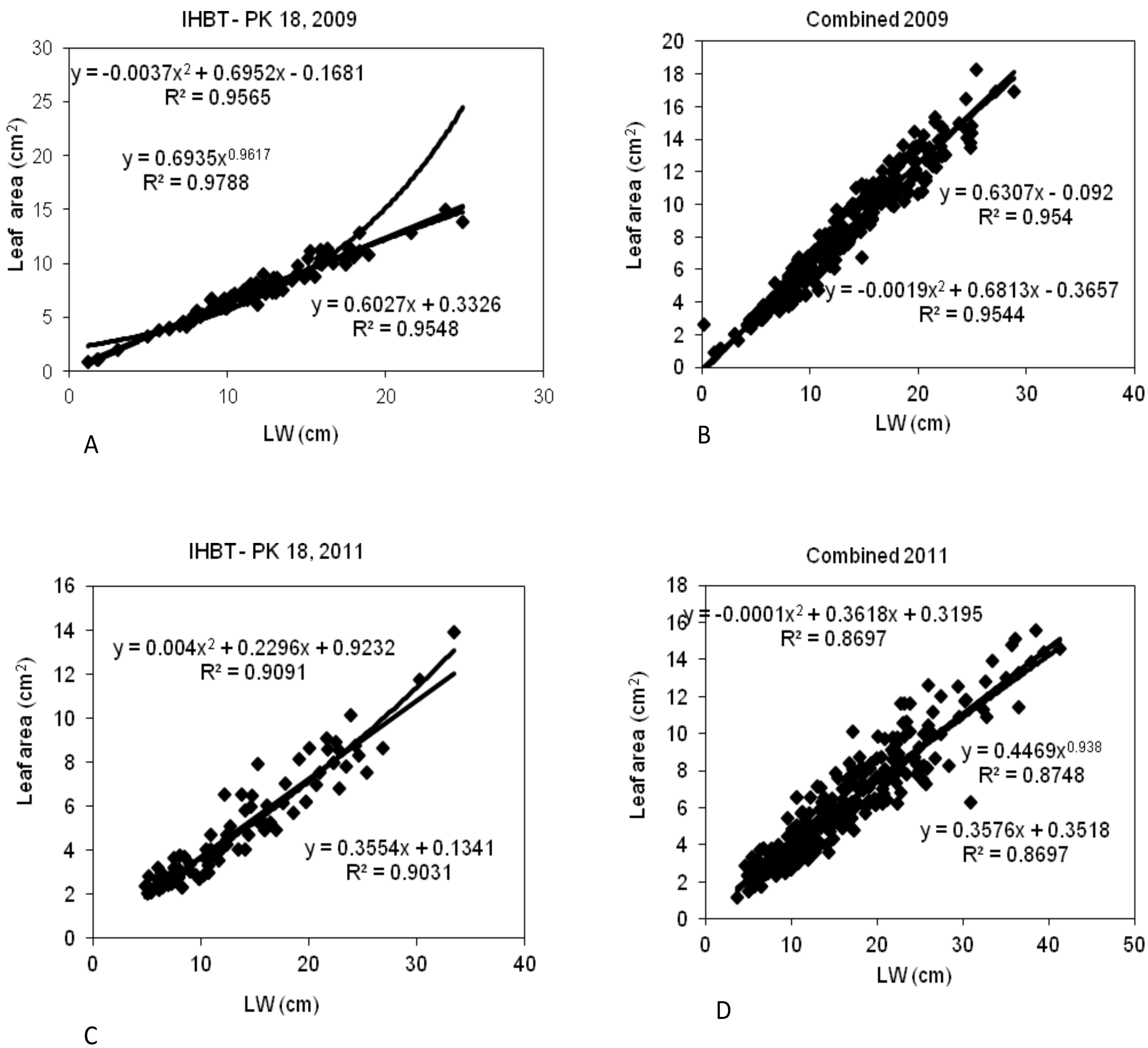

Figure 1. General regression models for estimating the leaf area of *P. kurrooa* from leaf length (L) and width (W) product.

coefficient of determination exceeds 0.85. Thus combining both criteria (high R^2 and low RMSE and CV), the linear model (y = 0.333 + 0.603LW, R^2 = 0.95, RMSE = 0.573, PRESS = 32.86, CV = 7.46%) can be proposed for LA estimation in *P. kurroa*.

Models based on LW have already been established recently for other medicinal and aromatic plants such as oregano (Caliskan et al., 2010), rose (Rouphael et al., 2010), sweet cherry (Demirsoy and Lang, 2010), green pepper (Cemek et al., 2011) etc. Though model based on single variable measurement offer the advantage of more efficient data correction, less complex calculation (Ne Smith, 1992) and require less time for leaf measurement. But in accordance with the suggestion of Ramesh et al. (2007) and Kumar and Sharma (2010), the measurement of both L and W can be more precise than estimates based on one dimension for leaf area estimation in stevia and clary sage, respectively. In our study, the combination of 'L' and 'W' showed higher precisions than as individuals either 'L' or 'W', therefore both parameters were necessary to estimate *P. kurroa* leaf area accurately.

The equations with lower R^2 and high CV were eliminated at the beginning of this study. Table 2 shows

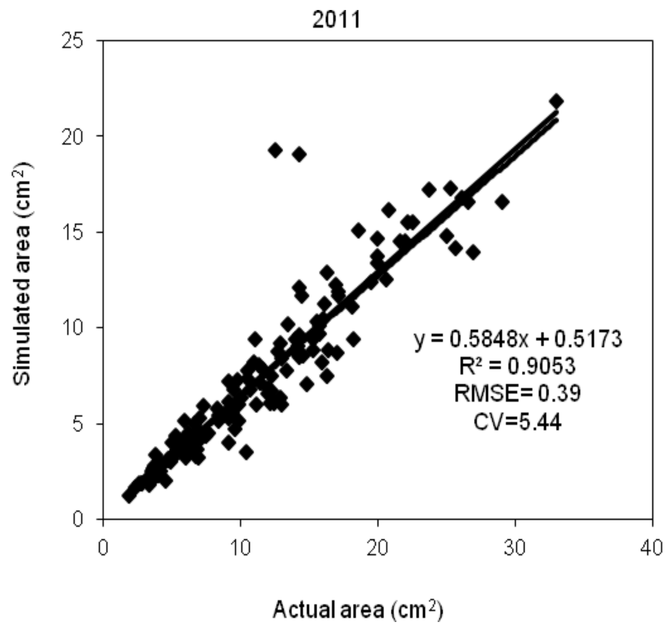

Figure 2. Validation of the actual versus simulated values of single leaf area (n = 150) using equation y = 0.333 + 0.603 LW, where 'y' is the individual predicted leaf area, 'LW' is the product of leaf length and width, solid line represents linear regression line of the model.

significant correlations were found between leaf dimension parameters with actual area. According to Lu et al. (2004), simple, linear relationships between leaf dimensions and LA would be preferable. In our work, the best-fitted curves between LA and leaf dimensions (L and W) were those of linear functions.

Conclusion

Results obtained from the present study demonstrated that *P. kurroa* leaf area could be predicted using simple linear measurements. Dimensions of the leaves can be easily measured in the field, greenhouse, or under natural habitat of the plant. Use of these equations would enable researchers to make non-destructive or repeated measurements on the same leaves and will also help in conservation of the plant. The leaf area predicted here based on linear dimensions agreed well with data from the crop at different ages. Since no models were previously developed for *P. kurroa* this work could be a valuable information towards *P. kurroa* leaf area estimation. With these developed models, researchers can estimate the leaf area of *P. kurroa* plants in physiological and quantitative studies accurately. In conclusion, the models derived in this study can be reliably used for estimating leaf area of *P. kurroa.*

ACKNOWLEDGEMENT

The authors are grateful to the Director, CSIR–IHBT, Palampur for providing necessary facility during the course of the study. The authors are also thankful to Mr. Sushil Kumar, technical assistant, for field management. Financial assistance from Council of Scientific and Industrial Research, New Delhi, India for undertaking this study is gratefully acknowledged.

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