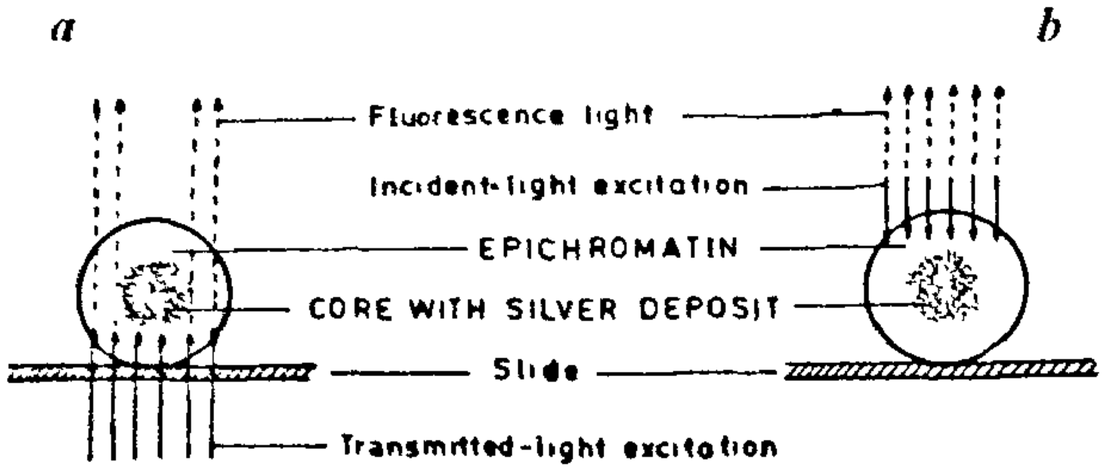

Figures 5. Diagrammatic representation of fluorescence from the cross-section of a silver-stained chromosome by two types of excitation *a*. In transmitted-light excitation, the silver deposition of the scaffold hinders the fluorescence light emitted by the epichromatin situated immediately above the scaffold making it visible *b*. In incident-light excitation, only the fluorescence light emitted by the epichromatin is visible and the scaffold remains invisible since it does not come in the way of either the incident or the fluorescence beam

chromatin fibres are possibly wrapped up by the scaffold protein, a portion of the chromatin remains exposed to the nucleoplasm. This may be equivalent to what Rattner[19] calls as the surface domain. Similarly the proteins of the scaffold along with SAR belong to the central domain. Thus it is concluded that the proteinaceous scaffold is situated longitudinally along the centre of the solid cylinder of epichromatin made of DNA-histone during all stages of chromosome cycle.

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Evaluation of growth behaviour of deodar and blue pine by using tree ring data from Uttarkashi, UP Himalaya

R. R. Yadav and A. Bhattacharyya

Birbal Sahni Institute of Palaeobotany, 53 University Road, Lucknow 226 007, India

The present study deals with the harmonic analysis of ring width series of deodar (1788–1987 AD) and blue pine trees (1826–1988) growing in Uttarkashi, UP Himalaya to understand short term fluctuations in productivity. Approximation of these tree ring series showed very good synchronization with cross correlation, 0.826 for *C. deodara* and 0.919 for *P. wallichiana*. Prediction of the series for 15 years by using this model indicated high values of similarity coefficient between predicted and original index values (64% for *C. deodara* and 71.4% for *P. wallichiana*). The results indicate that the dendrochronological series from the Himalayan regions could be used for short range predictions of tree growth.

FLUCTUATIONS in widths of tree ring sequences reflect the dynamics of wood growth dependent on environmental variables. It has been noted by several workers[1–9] that variations in tree ring widths differing in amplitude and duration are repeated more or less regularly over time. The cyclic components present in the tree ring time series have been used by several workers[9–14] for the prediction of the series to understand tree growth dynamics. Such predictions of the dendrochronological series would be useful in sustainable forest resource management.

In India variety of tree species growing in tropical and temperate regions are known to produce distinct growth rings. Studies so far conducted on some tree species from both the regions using ring width[15–21] and isotopic[22–24] variations have shown good dendroclimatic potential. With the development of the subject, besides its application in climatic studies variations in tree ring features such as thickness, density and chemical compositions have shown promise to assess how forests or individual trees respond to atmospheric chemistry, management and many other forest disturbances[25–29]. Application of such studies in various forestry aspects

has recently been taken up at Birbal Sahni Institute of Palaeobotany, Lucknow.

The management of the conifer forests in the Himalayan region is of growing concern for the country not only to preserve the Himalayan ecosystem but also to meet the increasing societal needs for timber, paper pulp, terpenes, resins, etc. Recent exploratory tree ring studies in the Himalayan region and establishment of a 745-year long deodar chronology from the UP Himalayas[20, 21] have shown a tremendous scope.

The present work deals with the harmonic analysis of dendrochronological series of deodar and blue pine growing in *terr-afirma* and mesic sites respectively in the Western Himalayan region and its applicability in prediction to understand short term fluctuations in the productivity of these trees.

Cedrus deodara (deodar or Himalayan cedar) grows throughout the Western Himalaya extending from East Afghanistan to Karnalli Valley in west Nepal at altitudes ranging from 1200 to 3000 m (ref. 30–32). Good winter snowfall, not too heavy monsoon and well drained soil seem to be of its primary ecological requirement. Good deodar forests are usually met in sites having rainfall from 1000 to 1750 mm. Its important associates are *Pinus wallichiana* and *Picea smithiana*, the former as a rule is found on the drier sites than the latter. Phenological information of deodar is not available, however, a preliminary growth climate relationship study of *C. deodara* has shown that the tree growth is favoured by cool and wet summers[18].

Pinus wallichiana (blue pine) trees are mostly restricted within latitudes 68° to 100°E along almost the entire length of Himalayas at an elevation of 1800–3000 m and sometimes ascending to 3700 m. Pine trees grow on a variety of geological formations but more preferably on well drained moist soils with an annual rainfall 1000–2000 mm. The trees grow in open and dense pure crop or in mixed forests mostly with deodar but also with *Abies*, *Picea*, *Taxus*, *Cupressus*, *Tsuga*, *Juniperus*, *Quercus* and *Rhododendron*.

For the present analysis core samples of wood were collected with the help of increment corer during two field trips, in October 1987 for deodar and in April 1989 for blue pine. Usually two cores were taken at breast height from each tree except in cases where the other side was not approachable due to steep rocky slopes. Twenty trees for each species were cored from each locality. These samples were collected from undisturbed stands with homogeneous habitat conditions. For sampling of deodar, a pure natural stand of mixed ages growing at an elevation of 2730 m was selected in Uttarkashi District in UP Himalaya. The trees were usually found growing perched on steep rocky slopes of south aspect with a thin sheet of soil. For pine comparatively young stands of mixed ages growing on mesic, north facing slopes at an altitude of 3000 m were selected. In the laboratory the tree cores were mounted on wooden frames for their easy and safe handling. Cross surfaces of core samples were polished with different grades of sand paper until the ring boundaries became very clear under the binocular microscope. Ring width patterns of various trees were then dated following the skeleton plot method of crossdating[33] to assign each ring to the calendar year of their formation. The outermost ring in *C. deodara* represents the year of collection, i.e. 1987 AD as the cores were collected during the end of October when growth of trees ceases in the Himalaya. But for *P. wallichiana* it was 1988, the preceding year of coring as the sampling was done in April when the wood growth was yet in offing. Width of dated growth rings was measured to the level of 0.01 mm accuracy. Measured ring width values were standardized to remove the long term trend which is assumed to be biologically caused. It was done by smoothing and correcting the measured values. Measured values were smoothed by using moving average method of 10 years filter length. The index for each tree was then calculated as the quotient of measured width to the value of smooth curve. Ring width indices were normalized by subtracting the overall mean and dividing by the standard deviation to remove tree to tree differences in variability. Normalized ring width indices were then averaged year by year to prepare the final chronology for further analysis. Eighteen tree cores of deodar and 23 of blue pine were used to prepare the chronologies of respective species. The deodar chronology extends from 1788 AD to 1987 AD whereas of blue pine from 1826 AD to 1988 AD (Figures 1 and 2).

The prediction of any time series is based on the presence of well-expressed cycles in the series which are stable in time. It is based on the principle to find the prominent cycles in the series which involve identification, approximation and extrapolation of the most important cyclic components. The method used earlier[10, 11, 14] has been applied here for the prediction of deodar and blue pine chronologies, the essential steps of which are summarized below.

The approximation of the normalized dendrochronological series $z(k)$ could be expressed as the summation of harmonical components

$$z(k) \approx \sum_{i=1}^{\infty} A_i \cos(2\pi f_i k) + B_i \sin(2\pi f_i k) \qquad (1)$$

where $k = 1$ to N years (the length of series), $\approx$ is the sign of approximation, f_i is the frequency of harmonical components, A_i and B_i are unknown parameters.

The sample spectrum was used to determine the most important harmonical components with which the approximation is done by means of variance. The sample spectrum shows the distribution of $z(k)$ variance by the harmonical components according to their frequencies. The spectrum of the series was calculated

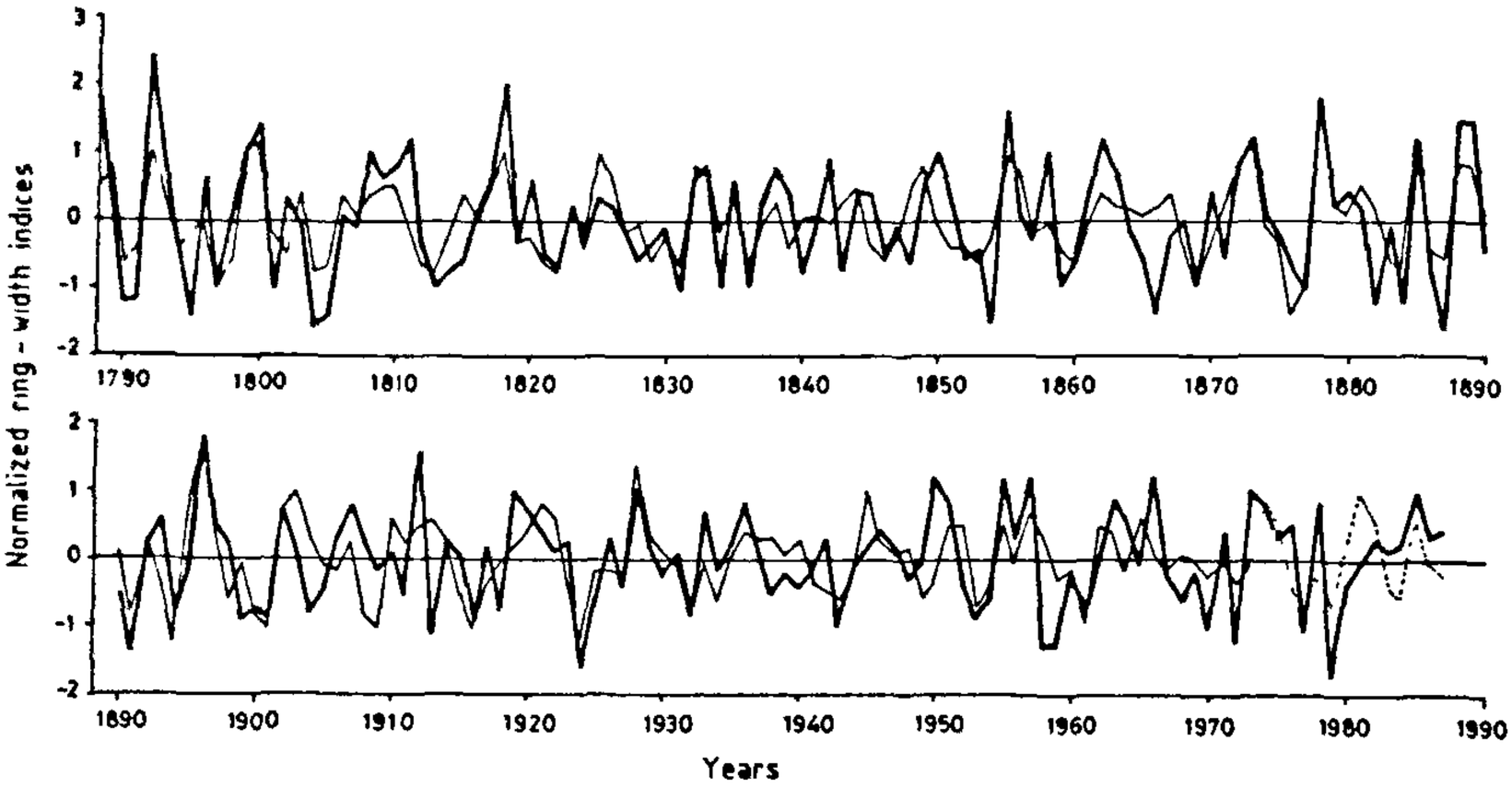

Figure 1. Ring width chronology of *Cedrus deodara* (1788–1987 AD) with approximated and predicted values

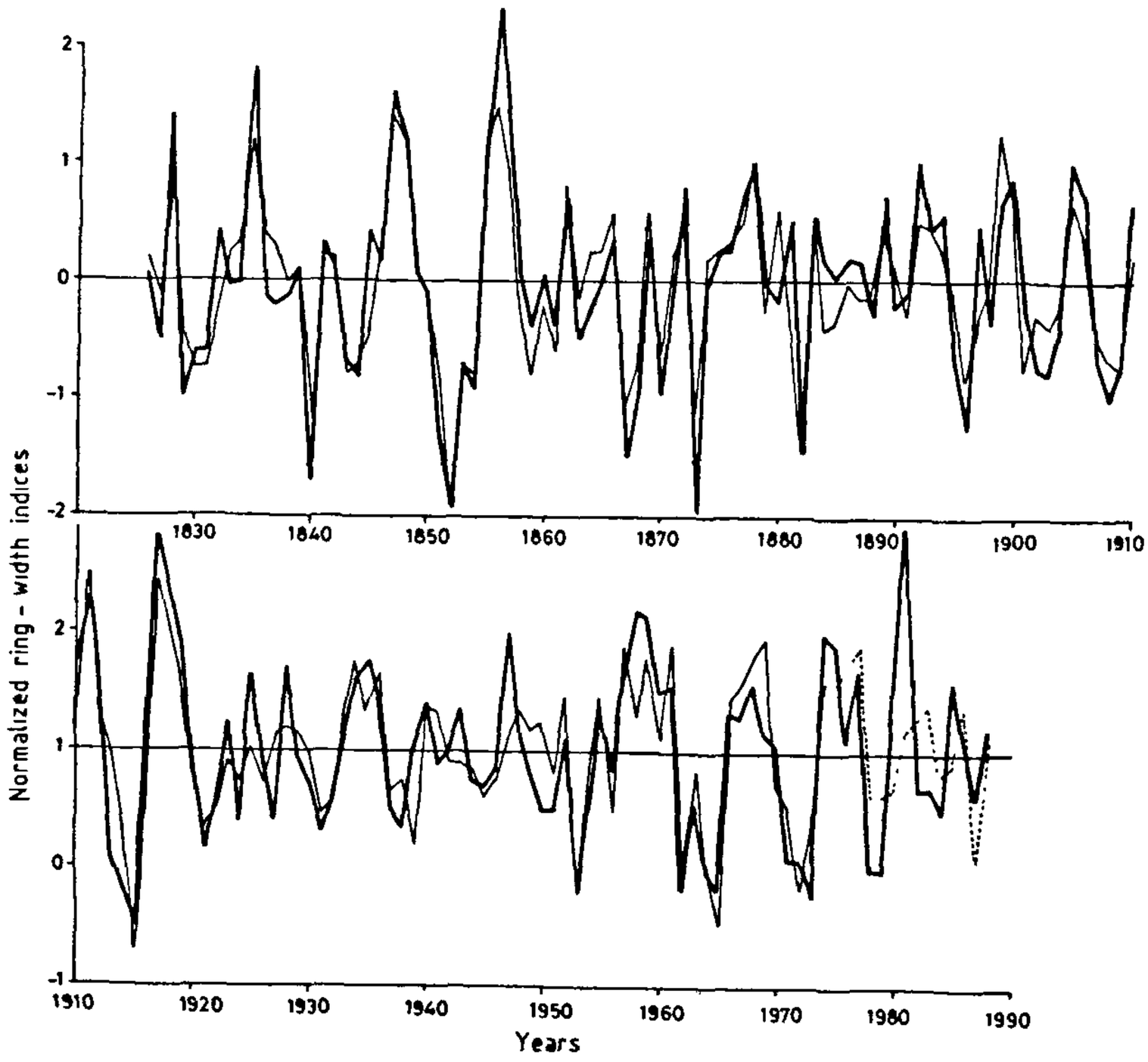

Figure 2. Ring width chronology of *Pinus wallichiana* (1826–1988 AD) with approximated and predicted values (thick lines, original, thin lines, approximated; broken lines, predicted index values).

by using the formula

$$R_{zz}(l) = 2\left(1 + 2\sum_{k=1}^{l-1} R_{zz}(k)\, w(k) \cos\frac{\pi l k}{F}\right), \qquad (2)$$

where $l = 0, 1, 2, \ldots, F$; $F = 2L$, where L is the length of the correlation function; $k = 1$ to N, where N is the length of the series; $w(k)$ is the spectrum smoothing window.

The maximum value of $R_{zz}(l)$ is calculated and the argument which agrees with this value is designated as $R(l^*)$ that is

$$R(l^*) = \max R_{zz}(l), \qquad (3)$$

the frequency which agrees with this value would be equal to

$$f_1^* = \frac{l^*}{2F} \cdot \tag{4}$$

The series was approximated by using the formula

$$A_1 \cos(2\pi f_1^* k) + B_1 \sin(2\pi f_1^* k). \tag{5}$$

A_1 and B_1 values are obtained by means of least square method by minimizing the sum of the squares of the differences of original and approximated values which could be expressed as

$$\sum_{k=1}^{N} (z(k) - (A_1 \cos(2\pi f_1^* k) + B_1 \sin(2\pi f_1^* k)))^2 \tag{6}$$

To avoid the error in the evaluation of f_1^* from the sample spectrum which is a discrete value, nonlinear regression model

$$A_1 \cos(2\pi f_1 k) + B_1 \sin(2\pi f_1 k) \tag{7}$$

was used and it was found to be more suitable for the approximation of the series. For evaluation of A_1, B_1 and f_1 parameters the method of Marquardt[34] was used in which the initial values of these parameters are used as 0, 0, f_1 respectively. When these values are calculated, the harmonic components are singled out from the series at every step and a new series is obtained. The sum of harmonical components which approximates the original series could be expressed as

$$z(k) \approx \sum_{i=1}^{l} \bar{A}_i \cos(2\pi \bar{f}_1 * k) + \bar{B}_i \sin(2\pi \bar{f}_1 * k), \tag{8}$$

where i represents the number of steps of harmonic components varying from 1 to l, the number of components chosen for approximation.

The level of approximation was adjudged by the degree of synchronization between original and approximated series. When from step to step difference in synchronization coefficients becomes very small, the procedure of singling out the harmonical components is completed and at the end absolute approximation of the original series is obtained. Forty components for *C. deodara* gave the best synchronization with cross correlation 0.86 between original and harmonized series (Figure 1). However, in case of *P. wallichiana* twenty components gave the best approximation with cross correlation 0.919 (Figure 2). High level of cross correlation between original and approximated series indicates that the developed model could be used for the prediction of the series. The predictions were made by using the same model by increasing the years k for the desired length using the above formula (8). In this case 15 years predictions were made (Figures 1 and 2). In order to verify the predicted values, the values of original and predicted series were also compared by using coefficient of similarity[35]. The synchronization coefficient for *C. deodara* was 64% and *P. wallichiana*, 71.4%.

Prediction of two dendrochronological series of *C. deodara* and *P. wallichiana* by using harmonic analysis has shown high values of similarity coefficients, i.e. 64% and 71.4% respectively for 15 years. This indicates that the cyclic components present in the dendrochronological series could be modelled for short range predictions. The predictions could further be improved by replicating large number of tree ring chronologies prepared from varied areas. The chronologies prepared from lesser number of samples include the effect of multiples of environmental and anthropogenic factors in the series which distort the cyclic components present in the series and thus intricate the approximation and prediction. By replicating the tree ring data effect of the desired climatic factor could be singularized and anthropogenic impacts deleted from the series. The replicated series with enhanced signal of single climatic factor would be more appropriate for prediction. As the growth periodicity greatly concerns the national resource requirement, advance information about the availability of forest resources would be very useful in evaluating the future sustainability of the biosphere system. Forestry practices could be accordingly oriented taking into consideration the future growth trend.

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Plant regeneration in barley through microtillering and multiple shoot differentiation

Sumita Kachhwaha and S. L. Kothari

Department of Botany, University of Rajasthan, Jaipur 302 004, India

Shoot multiplication was obtained through microtillering as well as multiple shoot formation in barley. Microtillering occurred from cultured immature embryos on MS and B5 media supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin, whereas from the cultured mature embryos shoot multiplication was through formation of *de novo* multiple shoot buds via an intervening callus phase. In the subsequent subcultures the number of shoots increased. Rooting of regenerated shoots was achieved on basal MS or B5 medium. Rooting was better on B5 medium in comparison to MS. The regenerated plants were transferred to field conditions where they flowered and set seeds.

PLANT regeneration in cereal tissue cultures can follow two different pathways, i.e. organogenesis or embryogenesis[1–6]. Organogenesis or shoot morphogenesis involves the development of *de novo* organization of shoot meristems in callus cultures. While somatic embryogenesis is the most common pathway of regeneration in most cereals, barley has been reported to be recalcitrant with regard to somatic embryogenesis or stable regeneration of plants through organogenesis[7, 8]. Microtillering and multiple shoot formation as a method of shoot multiplication has been described in sorghum[9–11], wheat[12, 13] and finger millet[14, 15]. This paper describes microtillering from immature embryos of barley and formation of multiple shoots in callus cultures of mature embryos.

Seeds of barley (*Hordeum vulgare* L. genotype BL-2) were obtained from the Agriculture Research Station, Durgapura, Jaipur. Seeds were field grown for immature embryo culture experiments. Young spikes 15 to 18 days after anthesis were harvested. Both the young spikes and the seeds were surface sterilized in 0.1% $HgCl_2$ (w/v) solution for 3–5 minutes and rinsed 3–4 times in sterile distilled water. While immature embryos ranging in size from 0.5 mm to 1 mm were gently excised immediately following several rinses with sterile distilled water, mature embryos were excised only after soaking the seeds in water for 48 h. The immature and mature embryos were cultured on MS medium[16] containing 3% sucrose and 0.8% agar (Qualigens) and B5 medium[17] with 2% sucrose and 0.8% agar. Both MS and B5 media were supplemented with 2.5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.5 mg/l kinetin. The cultures were incubated at 26 ± 2°C in the dark. Callus cultures of mature embryos with shoot buds were transferred to medium containing lower levels of 2,4-D in combination with kinetin for their multiplication. Elongated shoots were cultured on basal MS and B5 media. Thirty green plants were transferred to pots in the field directly without any previous hardening and all of them survived and grew to maturity.

After 10 days of culture, immature embryos formed 3–8 shoots on media containing 2.5 mg/l 2,4-D and 0.5 mg/l kinetin. Within 30–35 days the cultured immature embryos formed more than 8 shoots through