In conclusion, in the present study we have successfully induced adventitious roots from the leaf explants of *A. paniculata.* The adventitious roots were cultured in flask-scale suspension cultures using MS medium supplemented with 2.7 μM NAA and 30 g/l sucrose. Adventitious root cultures showed higher biomass as well as andrographolide accumulation capabilities. Our study demonstrates the possibilities of production of andrographolides for commercial purposes in a large scale using bioreactor cultures.


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# Climatic influence on radial growth of *Pinus wallichiana* in Ziro Valley, Northeast Himalaya

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**An attempt has been made here to study the climatic influence on variation of tree-ring width (radial growth) of Blue Pine (*Pinus wallichiana* A.B. Jackson) growing in five different sites in and around Ziro Valley, Arunachal Pradesh, Northeast Himalaya. The site chronologies have been evaluated to assess inter-site differences through several statistical analyses, viz. correlation matrices, principal component and hierarchical cluster analysis. Analysis of tree growth–climate relationship suggests that the pre-monsoon precipitation (December–April) is a significant factor influencing the growth of Blue Pine in all these sites.**

**Keywords:** Blue Pine, climatic influence, radial growth, tree ring.

BLUE Pine (*Pinus wallichiana*), a large evergreen conifer tree, is found all along the Himalayas from west Kashmir to east Arunachal Pradesh[1], at altitude generally ranging from 1800 to 3900 m. Typical habitats are mountain screes and glacier forelands and appears as a pioneer species, but it also forms old growth forests as a primary species in mixed forests with deodar (*Cedrus deodara*), spruce (*Picea smithiana*) and fir (*Abies pindrow*) in the temperate belt. In some places beyond 3000 m, it reaches the tree-line and is associated with birch (*Betula utilis*) and juniper (*Juniperus macropoda*)[2]. Blue Pine growing in several sites of the western and central Himalaya has been found suitable for various environmental and ecological applications in tree-ring analyses[3–9], but no such analyses are available from the northeastern part of the Himalaya.

In this paper an attempt has been made to analyse tree rings of *P. wallichiana* growing in and around Ziro Valley, Lower Subansiri District, Arunachal Pradesh, Northeast Himalaya, towards understanding its dendroclimatic potential from a new geographical region which is greatly influenced by the southwest monsoon.

Tree-ring samples of Blue Pine were collected from five forest sites characterized by open pine mixed broad leaved forest in and around Ziro Valley. These sites are Dobya (DOB), Hari (HAR), Hong (HON), Michi-Rant (MIC) and Raga (RAG; Figure 1). All these sites are

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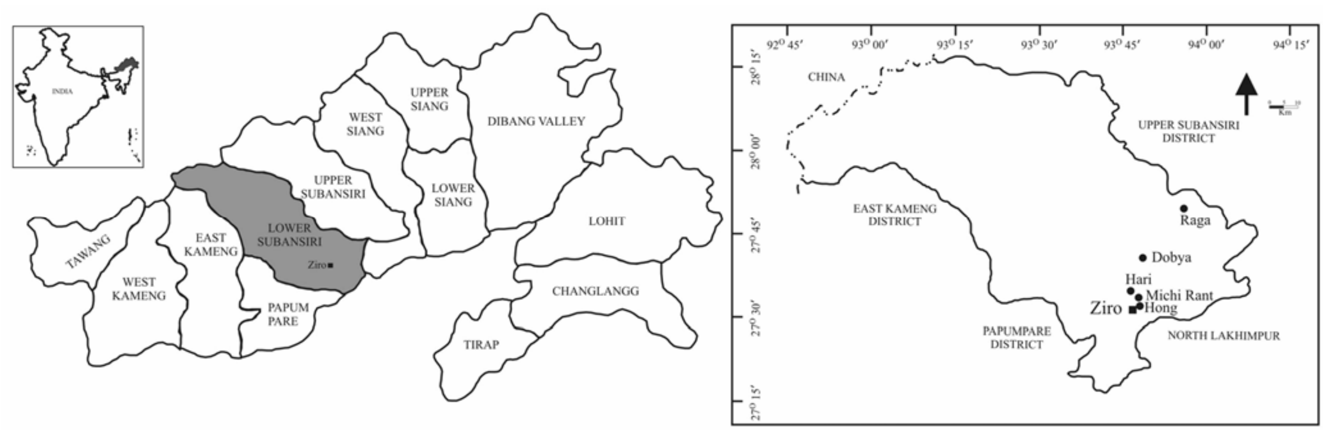


**Figure 1.** Map showing sampling sites of *Pinus wallichiana* at Ziro Valley, Lower Subansiri District, Arunachal Pradesh.

**Table 1.** Details of the tree-ring sampling sites from Ziro Valley

| Site | Site code | Altitude (m) | Number of trees and cores |
|---|---|---|---|
| Dobya | DOB | 1320 | 4 (11) |
| Hari | HAR | 1260 | 17 (36) |
| Hong | HON | 1245 | 3 (6) |
| Michi-Rant | MIC | 1255 | 15 (35) |
| Raga | RAG | 1582 | 6 (12) |

located at latitude and longitude ranging from 27°53′–27°68′ and 93°85′–93°89′ respectively. These sites are close to human settlements and thus highly disturbed. In HON village trees are confined to steep, rocky slopes, but most of them are found burnt by forest fire. Generally trees are young, except in some sites where several isolated, huge, girthed pine trees are recorded which have been preserved by the villagers in the memory of their ancestors. Otherwise, all old trees have been already logged. These protected trees, mentioned to be about 2000 years old by the local people, were planted by their ancestors after migrating from Tibet, and now these are identified by their names. Hundred cores from 45 trees of Blue Pine have been collected from these five forest sites with the help of Increment borer. Details of the tree-ring samples are given in Table 1.

All the tree cores of Blue Pine were mounted on a wooden frame. The surface of these cores was cut by the sharp razor blade and polished with the help of different grades of sandpaper to enhance the clarity of the cells. Tree rings of these cores were counted under stereo-zoom microscope and were dated to the calendar year of their formation using the typical skeleton plot technique[10]. The width of each dated tree-ring in each core was measured using VELMEX measuring system coupled with a computer. These measurements and dates were checked using the computer program COFECHA[11,12]. Cores that correlated poorly, even after rechecking and corrections (if there were any errors), were removed from the population prior to chronology development. In order to remove the gross growth trend largely unrelated to climate, and to remove effects of differential mean growth rates among individual trees prior to averaging into the mean chronology, standardization of each series was made. It was done by fitting a growth curve to the ring-width series and then dividing the measured value by the curve value at each year. After applying different detrending options for the standardization to check the improvement of chronology statistics, we have selected cubic spline which is appropriate for trees growing within the interior of the forests and not growing in stressed condition. The detrending for all the series was done using 30-year spline which retains 50% of the variance contained in the measurement series at a wavelength of 30 years. Two versions of chronologies, 'standard' and 'residual', were prepared for each site intended to contain a maximum common signal and minimum amount of noise. In the 'standard' chronology, detrending of measurement series was done first by fitting a curve to model biological growth trend to each series, and dividing out the growth model. The chronology was computed as a robust estimation of the mean value function to remove effects of endogenous stand disturbances; this enhances the common signal contained in the data. In the 'residual' chronology, autoregressive modelling was performed on the detrended ring measurement series. Robust estimation of the mean value function produces a chronology with a strong common signal and without persistence. The pattern of variation is generally similar among trees growing throughout the same region for the same time period because of variations in macro climatic factors, which are common to these trees[13]. Synchronization of growth pattern of Blue Pine in most of these sites covering a wide area of the Ziro Valley suggests a common force controlling the growth in the region. Generally, common growth pattern is exhibited in trees where climate has a significant role in limiting the tree growth. The averaging of standardized ring-width values or indices

**Table 2.** Selected statistics of tree-ring site chronologies of *Pinus wallichiana* at Ziro Valley

| | DOB | HAR | HON | MIC | RAG |
|---|---|---|---|---|---|
| Time-span | 1704–2000 | 1792–2000 | 1859–2000 | 1773–2000 | 1855–2000 |
| Number of years | 297 | 209 | 142 | 228 | 146 |
| Tr (Cr) | 4 (10) | 17 (28) | 3 (6) | 15 (35) | 6 (12) |
| MS | 0.182 | 0.161 | 0.207 | 0.137 | 0.147 |
| SD | 0.204 | 0.172 | 0.208 | 0.139 | 0.160 |
| AC (1) | 0.352 | 0.335 | 0.314 | 0.233 | 0.420 |
| Common period | 1865–88 | 1918–89 | 1904–2000 | 1837–98 | 1913–84 |
| Number of years | 124 | 72 | 97 | 162 | 72 |
| Tr (Cr) | 3(6) | 13(17) | 3(5) | 11(19) | 6(8) |
| Mean $r_{mt}$ | 0.234 | 0.176 | 0.183 | 0.163 | 0.183 |
| Mean $r_{wt}$ | 0.565 | 0.230 | 0.256 | 0.351 | 0.264 |
| Mean $r_{bt}$ | 0.151 | 0.174 | 0.165 | 0.153 | 0.176 |
| SNR | 1.832 | 3.642 | 1.124 | 3.907 | 2.021 |
| EPS | 0.647 | 0.785 | 0.529 | 0.796 | 0.669 |
| VARpc1 (%) | 36.5 | 24.6 | 36.6 | 21.9 | 29.2 |

Tr (Cr), Number of trees (number of cores); MS, Mean sensitivity; SD, Standard deviation; AC (1), Lag 1 autocorrelation; Mean $r_{mt}$, Mean correlation among all radii; Mean $r_{wt}$, Mean correlation within tree; Mean $r_{bt}$, Mean correlation between trees; SNR, Signal-to-noise ratio; EPS, Expressed population signal; VARpc1, Percentage of variance explained by the first eigenvector.

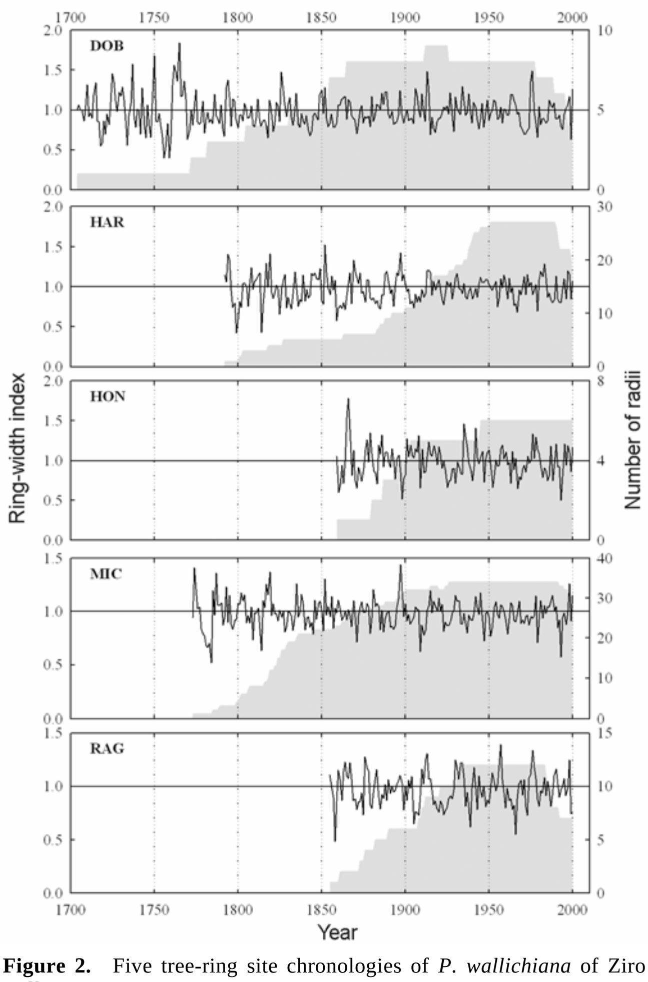


**Figure 2.** Five tree-ring site chronologies of *P. wallichiana* of Ziro Valley.

reduces the amount of variability due to non-climatic factors and enhances the ratio of climatic signal to non-climatic signal in the mean chronology. Among the five site chronologies (Figure 2) of Blue Pine in the Ziro Valley, the longest one was from DOB which extends from AD 1704 to 2000, whereas the shortest one (AD 1855–2000) was from RAG. The statistical performance of five tree-ring site chronologies is shown in Table 2. The poor statistical performance of all the five tree-ring chronologies may be due to poor replication of the sample size and site disturbances due to human influence. The chronology which has a low autocorrelation, a high mean sensitivity and a high standard deviation has been considered suitable for dendroclimatic analysis[13]. In the study sites, the mean sensitivity and standard deviation were the highest (0.207 and 0.208 respectively) at HON and lowest (0.137 and 0.139 respectively) in MIC. Autocorrelation was highest (0.420) in RAG and lowest (0.233) in MIC. The mean correlation within the trees and among all the radii was highest (0.565 and 0.234 respectively) in DOB, whereas mean correlation between trees is highest in RAG (0.176) and the value was almost the same in HAR (0.174). In general, signal-to-noise ratio was low in these chronologies. It ranged from 1.124 to 3.907, in which HAR and MIC were slightly better in comparison to the other three chronologies. A value of 0.85 has been put forward as a reasonable threshold for expressed population signal (EPS)[14], but none of these chronologies have crossed this value. The variances explained by HAR, MIC and RAG chronologies were much lower in comparison to the other two site chronologies, viz. DOB and HON (36.5 and 36.6% respectively).

Inter-site evaluations of the Blue Pine chronologies of Ziro Valley were pursued using three different statistical techniques: Pearson correlation matrix, principal component analysis (PCA) and hierarchical cluster analysis (HCA). These analyses help assess whether common variability exists within these site chronologies.

The Pearson correlation shows that the three sites, HAR, HON and MIC have good correlation with each other (at $P = 0.01$), but their correlation with the other two sites, RAG and DOB is low though in between later these two sites have good correlation (0.233 at $P = 0.01$; Table 3). Except two, the correlation among other chronologies was moderate. This may be due to the number of samples incorporated in the site chronology. These two site chronologies (HAR and MIC) showing good correlation (0.446 at $P = 0.01$) have maximum number of samples compared to the other three sites, where the number of samples is less (Table 1).

PCA assesses the degree of similarity and site-related differences among chronologies. It is a data reduction or structure detection technique, and is widely used in dendroclimatology[15]. The original variables (residual tree-ring chronologies) have been transformed into a new set of uncorrelated variables (eigenvectors or principal components) in such a way that a minimum number of components explains a maximum percentage of the variance in the dataset. For the present study, PCA was computed on the correlation matrix of all five ring-width chronologies of Blue Pine covering a common period of AD 1859–2000. The number of components retained for further analyses was determined on the basis of the eigenvalue trace (or scree plot). Two principal components were identified with an eigenvalue >1.0. These two components together explain 59.0% of the variance in the original dataset. Varimax rotation was used to aid in interpretation of the individual site loadings on each eigenvector[16]. Rotation of the principal components resulted in a clear separation of the sites (Table 4). It showed that 33.2% of the variance in tree-ring width was represented by HAR, HON and MIC in PC1 loading. Two sites, RAG and DOB fell in the PC2 loading, and both explained 25.8% of the total variance. Here RAG had the highest loading followed by DOB.

For HCA, Ward's method was employed using Pearson correlation coefficient as the measure of similarity. In the HCA dendrogram, two distinct groups (Figure 3) were identified which strongly agree with the natural orthogonal relationship noted in the correlation matrix (Table 3) and PCA (Table 4). Though it is not possible to establish the actual cause of this separation of the group, it seems to have resulted due to difference in the elevation of the tree-ring sites (low elevation 1245–1260 m and high elevation 1320–1582 m). However, a branch in the hierarchical tree suggests that these are from the same stock. This may be due to the year-to-year variability common in the major time-span between these two groups. This indicates that the signal change in between these chronologies seems to have resulted due to small-scale changes linked to the microclimatic conditions. This might to be related to the distribution of trees in relation to elevation rather than distance between these sites or there may be other causes which are yet to be established.

**Table 3.** Pearson correlation of five tree-ring site chronologies of *P. wallichiana* at Ziro Valley

| | DOB | HAR | HON | MIC | RAG |
|---|---|---|---|---|---|
| DOB | 1 | | | | |
| HAR | 0.146 | 1 | | | |
| HON | 0.167* | 0.293** | 1 | | |
| MIC | 0.212* | 0.446** | 0.241** | 1 | |
| RAG | 0.233** | 0.125 | –0.059 | 0.154 | 1 |

*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

**Table 4.** Summary of principal component analysis for the common period 1859–2000 of five tree-ring site chronologies of *P. wallichiana* from Ziro Valley

| | Principal component | |
|---|---|---|
| Site | PC1 | PC2 |
| HAR | 0.752* | 0.178 |
| HON | 0.737* | –0.168 |
| MIC | 0.695* | 0.304 |
| RAG | –0.079 | 0.860* |
| DOB | 0.250 | 0.633* |

*Rotated principal component with the highest loading for each chronology.

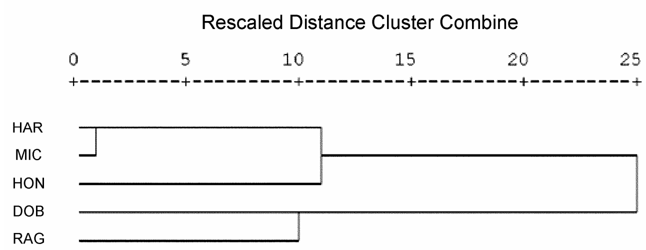


**Figure 3.** Dendrogram of *P. wallichiana* chronologies from hierarchical cluster analysis.

To evaluate the tree growth–climate relationship for a region, meteorological data (temperature and precipitation) from stations close to the sampling site are generally preferred, which could provide better understanding of climatic response to tree growth. These meteorological records are selected on the basis of their length, homogeneity and completeness of data. In the Northeast Himalayan region, stations having long climate records are not only limited in number, but most of them have high percentage of missing values thereby restricting their utility in qualitative dendroclimatic analyses.

The meteorological station, Ziro (93°42′E, 27°38′N, 1476 m asl), nearest to the sampling sites, has temperature and precipitation data extending from AD 1901 to 1980. Though it has long records, data for all the months during the years from 1963 to 1968 and 1977 are completely missing. Besides, more than six months' data are missing for the years 1901, 1962, 1971 and 1975. Overall for the period 1901–1980, the percentage of missing values in precipitation, minimum temperature and maximum temperature is 13.75, 14.58 and 13.65% respectively. In addition, there is a marked change in the quality of data; the variation in temperature was quite low in 1901–61 in comparison to 1969–80. This type of drastic change in climatic records generally happens when there is a shift of meteorological station from one site to other; but there is no such information for this station. Due to such discrepancies in these records, we prefer interpolated temperature and precipitation data (AD 1901–2000) of the nearest grid points (93°75′E, 27°75′N and 93°75′E, 27°25′N) of the CRU-TS 2.0 dataset[17]. The correlation between the annual temperature of the two grid points for the common period AD 1901–2000 was 0.982 ($P = 0.01$), whereas for precipitation, it was 0.959 ($P = 0.01$). Similarly, correlations for temperature and precipitation between data from meteorological station Ziro and average of two grid points for the common period AD 1902–1961 were 0.914 ($P = 0.01$) and 0.590 ($P = 0.01$) respectively. However, after eliminating the outlier value of 1952 from the precipitation dataset of the Ziro meteorological station, the correlation for the period 1902–51 was higher (0.837, $P = 0.01$). Time series of climate datasets from the meteorological station Ziro and average of two CRU TS 2.0 grid points along with bivariate plots for correlation are shown in Figure 4.

To assess the tree growth–climatic relationship of Blue Pine, bootstrap response functions[18] were performed between the principal component scores of tree-ring chronologies and the interpolated climatic data. The applicability of this technique has been established earlier by several workers in tree ring–climate modelling[19–22]. In this study climatic variables are taken for a 14-month period, i.e. from September of the previous year to October of the current year and the time-span has been taken from AD 1902 to 2000.

The response function of PC1 exhibited a positive response with February and April precipitation of the current year. The relationship was also positive with precipitation during December of the previous year and February of the current year in PC2 (Figure 5). Further, to understand the seasonal influences of precipitation on tree growth, we combined monthly climate data of some months which were found significant in limiting the growth of Blue Pine in this region, viz. December–February (DJF), February–April (FMA), December–April (DJFMA) and July–August (JA) in the computation of response function. In this analysis, among all these climatic variables, precipitation during pre-monsoon months, i.e. DJFMA was found highly significant. It has been recorded that the monsoon precipitation (JA) does not play a significant role in limiting the tree growth of this region. This is possibly due to the fact that the monsoon in this region never reaches the critical point to limit tree growth. In central Himalaya, Dolpo (western Nepal), growth of pine tree has also been found significantly correlated to the local summer rainfall, but not to the regional Indian Summer Monsoon Index[7]. Precipitation during pre-monsoon creates cool and wet conditions to recharge the soil moisture that can benefit the growth during the ensuing growing season, as it help the initiation of cambial activity and rapid development of early wood cells. In case of temperature, PC1 and PC2 have a direct relationship with the December and November respectively, of the previous year. This relationship suggests that significant photosynthesis can occur in the evergreen needles during warm winters, which is used as stored energy in the ensuing growing season[5].

The present tree-ring study of *P. wallichiana* at Ziro Valley, indicates that this taxon is suitable for dendroclimatic study for its clear and datable tree-ring sequences and synchronity in growth pattern. The climate signal (SNR) in these site chronologies of pine is moderate to low, which might have resulted due to intra-site difference. Generally, human disturbances and other natural calamities which vary from site to site may dilute the macro climatic signal, which is common to all these sites. These sites are highly disturbed due to clear-felling of trees for fuel and construction of houses, faulty agricultural practice ('jhum technique'), etc. By means of statistical analysis, viz. correlation matrices, PCA and HCA, two ecological zones for distribution of Blue Pine have been identified. This seems to be related due to the differences in elevation or changes in other site characteristics. A significantly positive response recorded in between tree growth and pre-monsoon precipitation indicates the significance of tree-ring data of Blue Pine not only in the pre-monsoon climatic reconstruction, but also in understanding of monsoon dynamics. Several studies have indicated that the

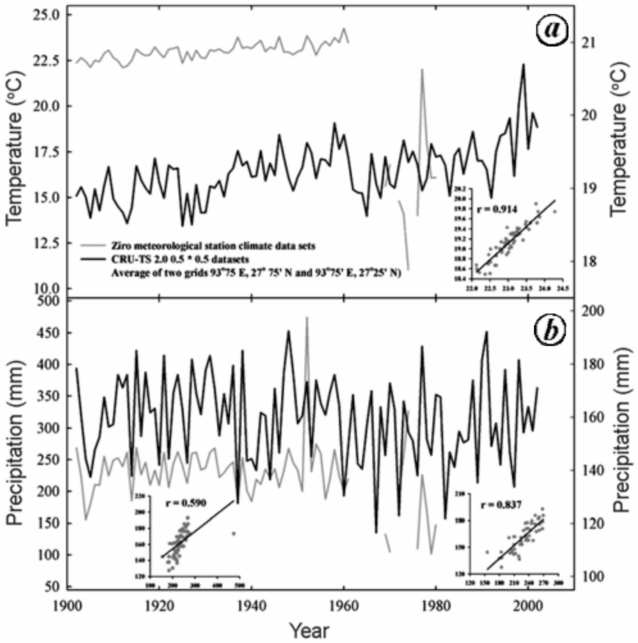


**Figure 4.** Comparison of climate data from meteorological station Ziro and interpolated CRU-TS 2.0 climate data. Bivariate plots for correlation.

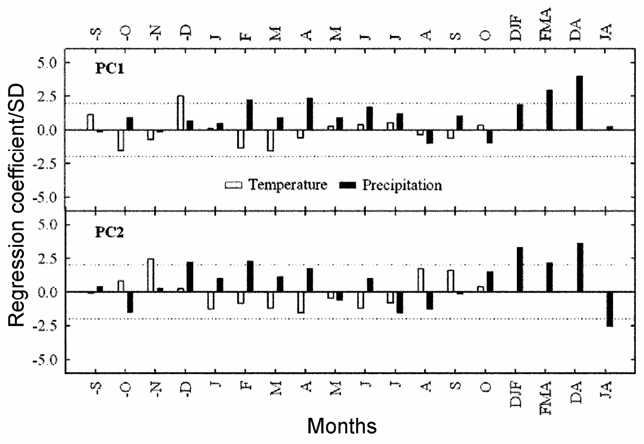


**Figure 5.** Response function plot of rotated principal component (PC) scores (PC1 and PC2) of *P. wallichiana* shown by regression coefficients divided by the standard deviation for the period 1902–2000. Dotted horizontal lines indicate significance level ($P < 0.05$) above and below the mean.

amount of precipitation in the form of snow during the previous winter and spring seasons regulates the build-up of the temperature gradient of the ensuing monsoon[23–28]. Thus the dendroclimatic analysis from this region has a great promise for the analysis of the long-term linkage of the different climatic systems linked to monsoon in the global perspective. However, to obtain statistically meaningful and significant relationship we need long, good replication of tree-ring data from the less-disturbed sites of this region. There is an ample scope in the collection of tree-ring samples in the form of sub-fossil woods, buried under the peat and sediments in the river bed, in several places of Ziro Valley, which not only provide a scope for the replication of data but also to extend pine chronology through cross-dating.


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