Variation in morphological characters of mycorrhizal seedlings of various provenances of *Pinus roxburghii* Sargent

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Abstract: The variation in growth of mycorrhizal seedlings of different provenances of *Pinus roxburghii* in terms of morphological and physiological characteristics have been observed under nursery conditions. Significant variation in growth characters i.e., shoot length, collar diameter, number of needles and root-shoot dry weight etc. were noticed through which Kalimath provenance was considered to be the best in respect to the growth in height (14.7 cm after six months and 43.7 cm after twelve months) and biomass production (2.15 gm seedlings^{-1} after six months; and 4.85 gm seedlings^{-1} after twelve months) respectively. Whereas Mayali provenance has yielded minimum values for these indices simultaneously. [New York Science Journal. 2010;3(2):1-8]. (ISSN: 1554-0200).

Key words Provenance, Germination, Mycorrhiza, Dry matter, Himalaya.

1. Introduction

Pines are found in a remarkably wide range of environments, from near the arctic, where winters are very cold and growing seasons are short, to the tropics, where frost never occurs and growth continues throughout the year (Knight et al., 1994). These are important and very often dominant components of vegetation over large part of Himalaya. Although relatively few pine species are found growing in the Himalayas, but they constitute ecologically significant and dominant vegetation in this area (Singh et al., 1994). *Pinus roxburghii* Sargent, commonly known as 'Chir-pine' is the most important pine among the six indigenous pine species of India, which is much valued for its timber and oleoresin. It occurs in the monsoon belt of the outer Himalaya, from North – eastern part of Pakistan to Arunachal Pradesh in India at elevations varying from 450 to 2300m asl. It is found distributed over a long strip of about 3,200 km between latitudes 26°N to36°N and longitudes 71°E to 93°E. The species is economically very important and is used for variety of purposes viz., timber for house construction, fuel wood extraction, charcoal formation, resin tapping, needles for fuel briquetting, cattle bedding and for manufacturing organic manure, etc.

The natural regeneration of different plant species through seeds depends upon the production and germination capacity of the seeds and the successful establishment of the seedlings. The success of any plant or group of plants in different regions depends upon the adaptability of roots to the various environmental regimes (Pavlychenko, 1937). Further several problems related to the choice of species and seedling establishment remain unresolved, until details of the root system of the species concerned are studied (Bhimaya, 1965). However, difficulties and expense involved in the excavation of the roots are primarily responsible for the inadequacy of research in this field. Work on seed testing of various provenances of *Pinus roxburghii* from Uttarakhand and Himachal Himalaya has been done by Sharma et al. (2001), Ghildiyal et al. (2007, 2008, 2009) and Ghildiyal and Sharma (2005, 2007), whereas, studies on effect of mycorrhizae on *Pinus roxburghii* have been studied by Ghildiyal (2003) and Rawat and Sharma (2008).

The symbiotic association of plant roots with soil fungi called mycorrhiza is now quite well known. Frank in 1885 first coined the term 'mycorrhiza' meaning fungal root. Functionally mycorrhiza represents symbiotic association between the plant root and non-pathogenic soil fungi which both mutually benefit the patners due to bi-directional flow of nutrients. Mycorrhiza confers many attributes to plant, such as growth stimulation due to increased nutrients uptake, tolerance of plants to odd conditions and bio control of root diseases etc. The genus Pinus is mucorrhiza dependent species, which plays an important role in establishment and survival of plants in nature. In the natural zone of pines, introduction of mycorrhizae is not required as the soil is infested with mycorrhizal fungi. The deficiency of mycorrhizal fungi arises when pines are raised outside their geographical distribution (natural range), and in such cases introduction of mycorrhizae is a must, especially in the nursery stage to ensure the survival of the seedlings. Taking it into consideration, this paper reports the results of the performance of mycorrhizal seedlings of various provenances of *Pinus roxburghii* Sargent, in relation to different morphological and physiological characteristics after 6 and 12 months of their growth under nursery conditions.

2. Material and Methods

The study was conducted on the germination behaviour of mycorrhizal and non-mycorrhizal seedlings of *Pinus roxburghii* after collecting the seeds from 16 provenances, which were distributed in 4 districts i.e., Pauri, Chamoli, Rudraprayag and Tehri of Uttarakhand Himalaya (latitude 29° 47′ to 30° 02′ N and longitude 78° 32′ to 79° 28′E). The majority of rain fall (705mm to 1890mm) in these regions occur during monsoon period i.e., from June to September and area is represented by sub-tropical to temperate climates. The detailed geographical and meteorological attributes of various provenances are given in Table. 1 and Figure. 1.

Table 1. Geographical and meteorological descriptions of the seed sources of *Pinus roxburghii.*

Provenance	District	Latitude (N)	Longitude (E)	Altitude (m)	Temperature(°C)		Mean annual rainfall (mm)
					Min.	Max.	
Ashtavakra	Pauri	30° 13'	78° 48'	960	5.76	37.70	705.00
Augustmuni	Rudraprayag	30° 23'	79° 02'	875	4.31	36.59	833.00
Badiyargarh	Tehri	30° 17'	78° 50'	1080	7.50	36.30	930.00
Ghansali	Tehri	30° 27'	78° 39'	890	5.00	34.60	1230.00
Godnar	Chamoli	30° 30'	79°16'	1680	1.30	24.00	1890.00
Jaiharikhal Jasholi	Pauri	29° 47'	78° 32'	960	7.54	37.00	1150.00
Kalimath	Rudraprayag	30° 16'	79° 04'	1520	1.60	34.10	1025.00
Lansdowne	Chamoli	30° 34'	79° 05'	1540	1.60	26.10	1257.50
Mayali	Pauri	29° 50'	78° 41'	1703	-0.90	25.80	1260.00
Pabo	Tehri	30° 23'	78° 47'	1400	2.60	25.10	1030.00
Pauri	Pauri	30° 15'	79° 01'	1640	1.8	32.4	875.00
Pokhal	Pauri	30° 09'	78° 48'	1660	-0.48	26.30	1792.00
Tangni	Tehri	30° 25'	78° 59'	820	5.70	37.63	800.00
Thalisain	Chamoli	30° 29'	79° 28'	1480	4.20	25.50	990.00
Vana	Pauri	30° 02'	79° 03'	1640	1.9	31.00	1025.00
	Chamoli	30° 38'	79° 05'	1610	1.30	24.00	1660.00

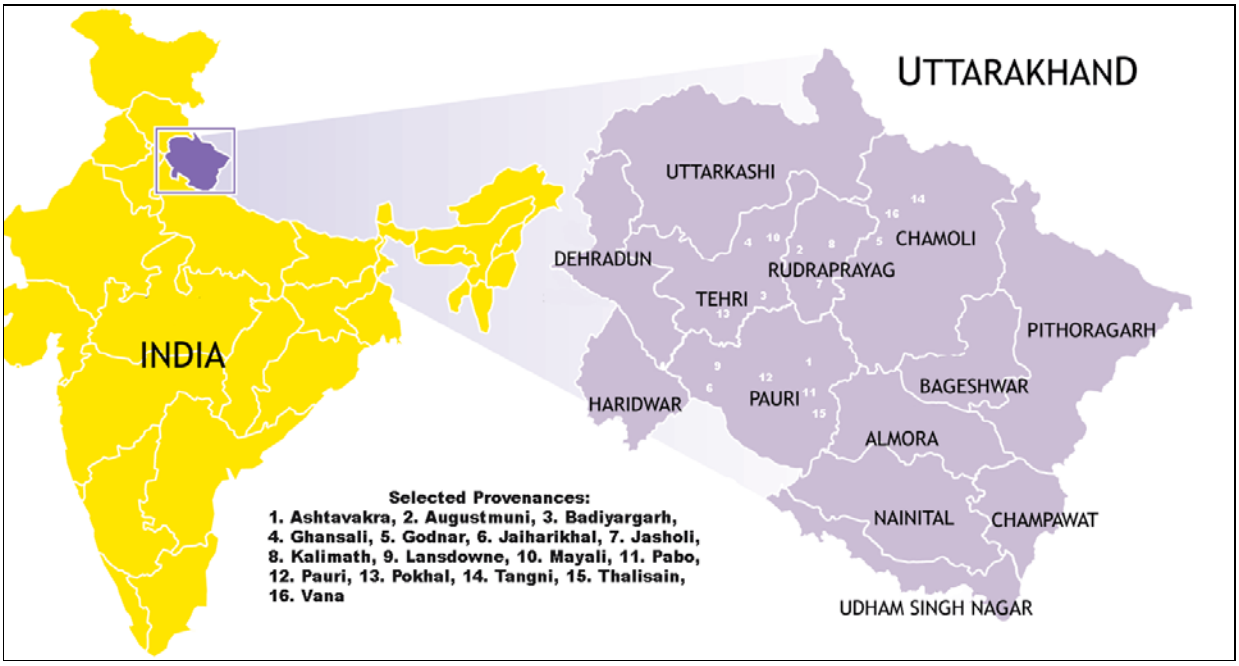

Figure 1. Map of the Study areas.

The effect of mycorrhizal inoculation on the growth and development of the seedlings of *Pinus roxburghii* was assessed under polyhouse conditions. The soils from two quite distinct disturbed places were collected from 0-15cm depth, and were filled in the earthen pots of nearly same dimensions. The total numbers of filled pots were subjected to inoculation with ectomycorrhizal fungi. For another experiment; to observe the effect of mycorrhizae on the growth and development of the seedlings of chir-pine, 10 seeds of each provenance were sown directly on the earthen pots, containing the soil, taken away from healthy chir-pine forest, presuming sound growth of indigenous mycorrhizal propagules. After 3 weeks of germination, the pots were maintained with five seedlings each. The inoculation was again supplemented to ensure the infestense by mycorrhizae. This was done by adding 3gm of fresh ectomycorrhizal inoculum, consisting of short roots and infested soil, which was derived from healthy chir-pine forest. The seedlings were irrigated, as and when required. The observations were recorded after every two months interval on various growth parameters. The mycorrhizal formation in the root zone was evaluated simultaneously by counting the number of dichotomous branching formed by the root laterals after each time interval.

Observations with respect to height, collar diameter, number of needles on mycorrhizal and non-mycorrhizal seedlings were taken after six and twelve months. The study pertaining to length of tape roots, number of lateral roots and biomass production was conducted after removing them from the polythene bags, and washing the root system with water. Shoot portion of each of the selected seedling was cut at the collar height and fresh weight of the shoot and root was recorded separately. They were further dried inside an oven at 70°C till a constant weight, for determination of biomass.

The mycorrhizal root infection percentage was assessed by clearing and staining of root bits as per procedure given by Phillips and Hayman (1970), whereas the quantification of mycorrhizal infection was recorded by grid-line intersect method, given by Giovanetti and Mosse (1980) to quantify the root infection. Stained root samples were spread evenly on a plastic petridish, scribed with grid lines on the bottom of the petridish. After uniform spreading of root bits, grid lines were scanned under dissecting microscope and the total number of root intersections with grid as well as the number of intersects with colonized roots were recorded. The questionable root colonization was verified under compound microscope. Total and colonized root length (R) was calculated using the formula: $R = An/2H$ Where, A is the total area in which roots were distributed and n is the number of intersections. When the interline distance is 1.27 cm, then PA = 2H and total colonized root length in cm equals the number of total or colonized root intersections with grid lines.

3. Results and Discussion

The seedlings of all the 16 provenances which were maintained in the nursery and measured for their height, collar diameter, root length, number of needles produced and number of lateral roots developed at the intervals of six and twelve months have indicated significant variations in their seedling parameters (Table. 2 and Figure. 2, 3).

The mycorrhizal seedlings of all the provenances, when measured after six months of growth, the maximum height (14.7 ±0.18 cm), collar diameter (0.86 ±0.01 cm), number of lateral roots (74.0 ±2.42), Shoot biomass (1.18 ±0.02 gm/seedling) and root biomass (0.97 ±0.03 gm/seedling) were registered for the Kalimath provenance. On the other hand minimum values for height (10.5 ±0.18 cm), collar diameter (0.38 ±0.03 cm), root length (7.5 ±0.13 cm), number of needles (152.0 ±1.29), number of lateral roots (43.0 ±2.50), shoot biomass (0.59 ±0.02 gm/seedling) and root biomass (0.30 ±0.01 gm/seedling) were recorded for Mayali seed source. Similarly after twelve months (one year) of growth, the height of the mycorrhizal seedlings ranged from 30.0 ±1.58 to 43.7 ±1.85 cm; collar diameter from 0.62 ±0.07 to 1.29 ±0.04 cm; the root length from 25.8±4.28 to 48.8 ±3.12 cm.; the number of needle from 457.0 ±19.03 to 698.8 ±14.53; number of lateral roots from 75.4 ±1.42 to 103.0 ±1.21 respectively, in various provenances (Table.2). The root and shoot biomass production after twelve months was observed as maximum (1.98 ±0.90 and 2.87 ±0.56 gm $seedling^{-1}$ respectively) for Pabo and Kalimath provenances, whereas minimum (1.09 ± 1.47 and 2.02 ±0.28 gm $seedling^{-1}$ respectively) for Vana and Tangni provenances in mycorrhizal seedlings (Figure 2 and 3).

3.1 Mycorrhizal Colonization

Significant effects of seed source on ectomycorrhizal colonization have been observed in seedling roots, which are presented in Table. 2. The range of ectomycorrhizal colonization varied between 38.17% to 59.99% in various provenances, after one year of growth. Maximum ectomycorrhizal infection was observed in the seedling roots of Kalimath seed source, (59.99%). Poor root colonization by ectomycorrhizal infestation was observed in seedlings of Mayali provenance (38.17%). In other seed sources (except Jasholi, Pabo and Jaiharikhal) almost 50 per cent seedling roots had ectomycorrhizal colonization.

The data presented in Table. 2 reveals that ectomycorrhizal colonization varied significantly among seed sources and the intensity of infestation increased with time. Seedlings of different seed sources, which had maximum infestation at six months were consistently better after one year of growth also, and the trend of variation was similar.

Table 2. Seed source variation in relation to mycorrhizal colonization and production of total seedling biomass after 6 months and 1 year of age.

S. No. Provenance	Mycorrhizal root infestation (%)		Total root length after infestation (cm)		Number of spores/ gram soil		Total seedling dry weight (g/seedling)	
	6 Months	1 Year	6 Months	1 Year	6 Months	1 Year	6 Months	1 Year
Ashtavakra	33.62	50.72	10.20	38.60	3.34	4.82	1.25	4.17
Augustmuni	30.16	47.07	8.40	25.20	2.72	6.41	0.99	4.04
Badiyargarh	28.58	45.72	6.40	27.50	1.32	2.70	1.13	4.11
Ghansali	30.62	47.31	7.00	31.00	2.25	4.36	0.95	4.25
Godnar	34.76	54.61	8.60	36.00	3.84	7.62	1.43	4.50
Jaiharikhal	32.69	49.24	8.20	23.80	2.65	4.56	1.24	3.36
Jasholi	32.72	49.21	9.80	30.80	3.40	6.66	1.20	4.06
Kalimath	38.44	59.99	10.20	42.60	4.92	8.40	2.15	4.85
Lansdowne	35.24	56.37	8.60	26.20	3.44	5.25	1.44	3.6
Mayali	24.88	38.17	6.00	21.60	1.18	2.42	0.89	3.26
Pabo	32.92	49.36	9.30	41.80	1.96	2.96	1.46	4.72
Pauri	36.48	56.92	11.80	32.40	3.78	6.17	1.48	4.19
Pokhal	28.82	46.84	11.80	32.40	2.61	5.56	1.03	4.12
Tangni	32.05	48.02	8.10	25.30	2.96	7.14	1.54	3.83
Thalisain	34.54	54.42	11.10	37.40	2.86	5.27	1.61	4.07
Vana	31.56	47.42	8.80	29.80	2.52	4.64	1.37	3.32
Mean	32.38 ±0.832	50.09 ±1.332	9.02 ±0.436	31.40 ±1.606	2.86 ±0.237	5.31 ±0.433	1.323 ±0.078	4.028 ±0.116
Range Min.	24.88	38.17	6.00	21.60	1.18	2.42	0.89	3.26
Max.	38.44	59.99	11.80	42.60	4.92	8.40	2.15	4.85
C.D. at 1%	5.40	6.20	1.20	6.80	0.79	0.94	0.42	0.82

Table 3. Simple correlation between mycorrhizal seedling parameters

	Variables	1	2	3	4	5	6	7	8	9	10
1	Shoot biomass	1.000									
2	Shoot length (cm.)	0.512*	1.000								
3	Collar diameter (cm.)	0.819**	0.621*	1.000							
4	Number of needles	0.527*	0.448	0.519*	1.000						
5	Root biomass gm/seedling	0.434	0.386	0.536*	0.328	1.000					
6	Root length (cm.)	0.802**	0.681**	0.799**	0.678**	0.636**	1.000				
7	Number of lateral roots	0.558*	0.402	0.575*	0.593*	0.199	0.628**	1.000			
8	Mycorrhizal root infestation (%)	0.645**	0.361	0.634**	0.656**	0.279	0.588*	0.554*	1.000		
9	Total root length after infestation (cm)	0.804**	0.661**	0.810**	0.639**	0.615*	0.989**	0.651**	0.590*	1.000	
10	Number of spores/ gram soil	0.222	0.205	0.466	0.482	0.399	0.275	0.221	0.648**	0.272	1.000
11	Total seedling dryweight (gm/seedling)	0.798**	0.518*	0.774**	0.487	0.889**	0.833**	0.416	0.514*	0.819**	0.379

**. Correlation is significant at the 0.01 level.

*. Correlation is significant at the 0.05 level.

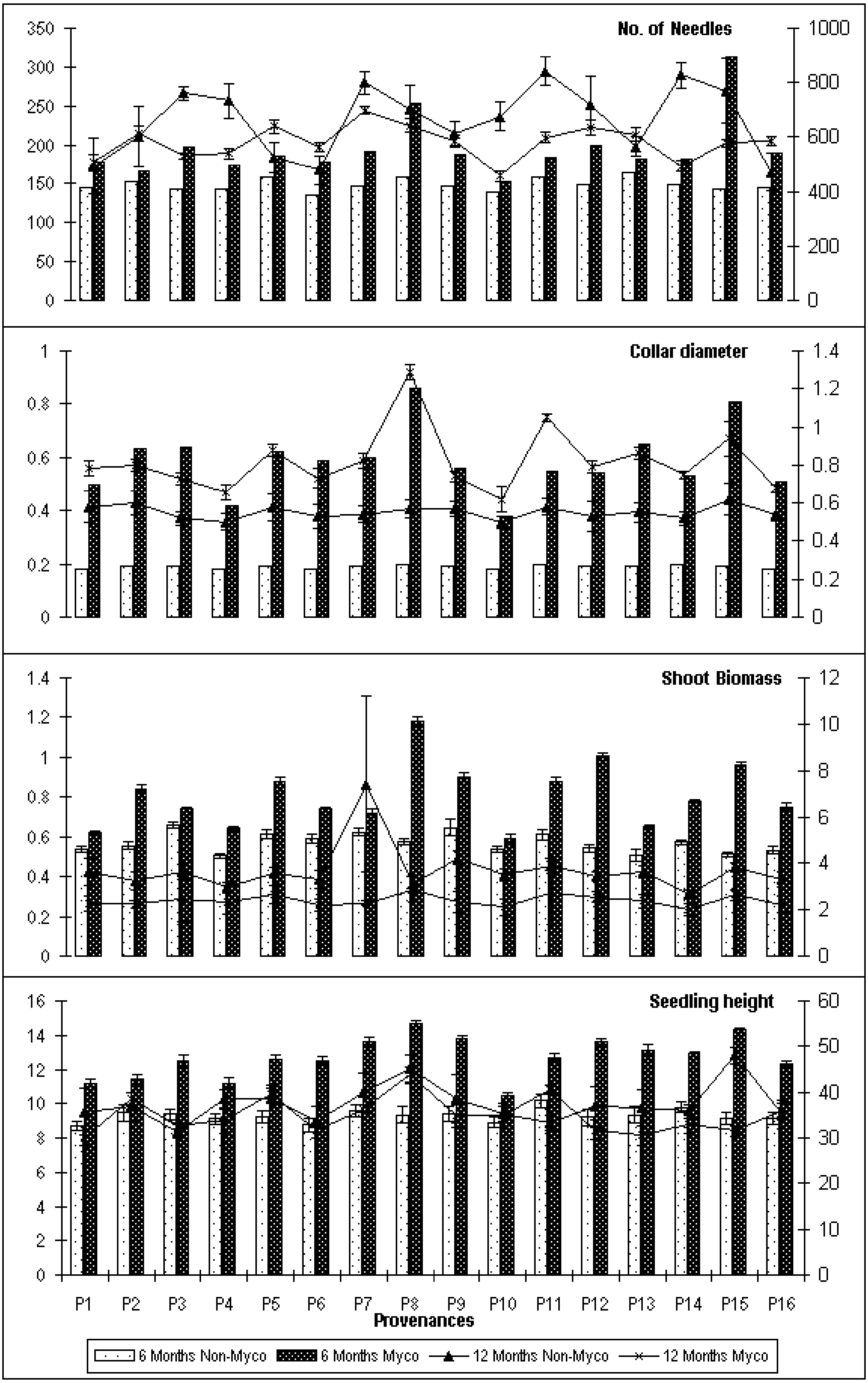

Figure 2. Comparison between mean morphological characters and biomass production of non-mycorrhizal and mycorrhizal seedlings.

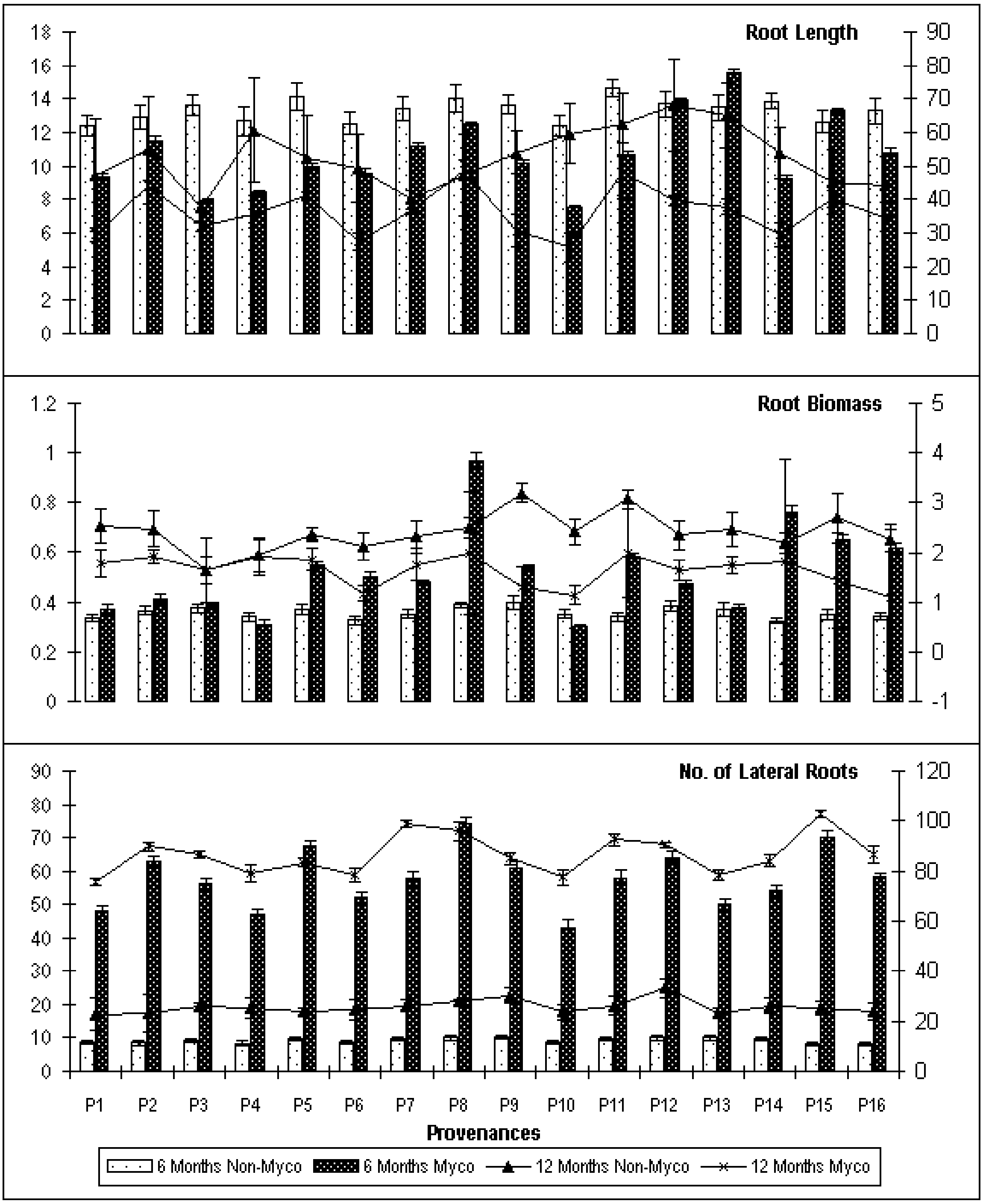

Figure 3. Comparison between different root parameters of non-mycorrhizal and mycorrhizal seedlings.

3.2 Ectomycorrhizae induced root length

Ectomycorrhizae induced root length varied significantly (P = 0.01) among seed sources and the trend of variation was similar to ectomycorrhizal colonization (Table. 2). Seedlings of Kalimath seed source had maximum root length (42.60 cm), while the minimum root length was observed in the seedlings of Mayali (21.60 cm) seed source. Jaiharikhal (23.8 cm) and Agustmuni (25.2 cm) seed sources also had low root infection and the values were statistically equal to minimum total root length value. Seedlings roots of Godnar (36.0 cm), Pauri (32.4 cm) and Pokhal (32.4 cm) seed sources also had more root lengths due to heavy ectomycorrhizal infestation. Most of other seed sources (expect Tangni, Lansdowne and Badiyargarh) represented intermediate group of root length, based on seedlings root infestation after one year of growth (Figure 3).

3.3 Number of ectomycorrhizal spores per gram of soil

It is evident from the data presented in Table. 2, that the amount of variation observed in this parameter is narrow. The range of number of spores/gram of soil varied from 2.42 to 8.40 spores/gm of soil after one year of inoculation, indicating maximum number of spores in the soil contained by seedlings of Kalimath seed source (8.40 spores/gm of soil). Soils sustaining Tangni, Agustmuni and Jasholi seed sources grown seedlings had comparable spore counts. The minimum value (2.42 spores/ gm of soil) was recorded in the soil containing Mayali source seedlings. Another group of seed sources, comprised of Pokhal (5.56 spores/gm of soil), Lansdowne (5.25 spores/gm of soil) and Thalisain (5.27 spores/gm of soil) were comparable with each other and found containing comparatively higher number of spores than poor and intermediate performers, after one year of development.

3.4 Total seedling dry weight

The analysis of variance in total seedling dry weight has manifested statistically significant differences among seed sources in the mycorrhizal seedlings after 6 months and one year of development. The range of total seedling dry weight after six months of growth varied from 0.89 gm/seedling for Mayali seed source to 2.15 gm/seedling for Kalimath seed source. After one year of growth, the seedlings raised from Godnar (4.50 gm/seedling), Ghansali (4.25 gm/seedling) and Pabo (4.72 gm/seedling) seed sources produced total seedling biomass comparable to maximum value (4.85 gm/seedling by Kalimath provenance). However, the seedlings of the Mayali seed source (3.26 gm/seedling) produced minimum total seedling dry weight after one year of development.

The increase in the survival of mycorrhizal pine seedlings throughout the first observation has clearly been evidenced from Table. 2. At the beginning of the 60 days test, all seedlings had approximately the same number of feeder roots. There is a marked variation in growth of mycorrhizal pine seedlings after two, four, six, eight, ten and twelve months under nursery conditions. The morphological characters such as height, collar diameter and some other characteristics are known to be strongly inherited (Shivkumar and Banerjee, 1986). Therefore, seedlings height may be considered as one of the most useful characters for early selection of superior provenances. However, selection of seed lots on the basis of one character alone may not give some time the desired level of superiority as the non-mycorrhizal seed source, which sometimes show maximum seedling height (e.g. Badiyargarh provenance), but its seedling dry weight was very poor. This has shown that seedling source selection should be on the basis of multi-trait consideration, which has also been emphasized by Vakshasya et al. (1992).

Taking into consideration the importance of morphological and physiological seedling characteristics, it appears that the provenance of Kalimath area in District Rudraprayag was outstanding in its performance among all the provenances studied followed by Thalisain, Pabo and Pauri provenances. Mayali and Ghansali provenances were observed as comparatively weaker in growth and biomass production. The mycorrhizal seedlings have shown marked differentiation in respect to the various seedling traits.

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