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RESEARCH ARTICLE

Studies on relationship between Stomatal density and oleoresin yield in Chirpine (Pinus roxburghii Sargent)

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Abstract

The production of oleoresin has suffered from past and has come down from 75 thousand tones in 1975 to 25-30 thousand tones in 1995 (Coppan and Hone, 1995). In many parts of the world, considerable work has been done on pine species other than chirpine. So there is a need of hour to increase the oleoresin production potential from chirpine trees to narrow down the gap between the requirement and supply. This can be achieved either through horizontal spread of chirpine or vertical improvement of the species through selection of high resin yielding progenies for advanced generation development. As there is very less scope of horizontal improvement due to population pressure, so the only method is the genetic improvement which can be conventionally achieved through selection of high resin yielders among the progenies of plus trees. The present Studies on relationship between Stomatal density and oleoresin yield in Chirpine is a step forward in this direction. Dibkon-P3 and Leda-P5 progenies recorded with maximum number of stomatal rows on round surface. Whereas, maximum number of stomata per mm of a row was obtained in Bagthan-PT-Black Top. Correlation studies between oleoresin yield and number of stomata per mm of a row of progenies projected significant and positive correlation. Genetic advance (320.28) and Genetic gain (35.51%) were found to be highest for oleoresin yield. Number of stomata per mm of a row and Average number of stomatal rows on needle surface had significant effect on the resin yield; hence oleoresin yield can be predicted on the basis of these characters

Introduction

Pinus roxburghii Sargent, commonly known as Chir Pine belonging to family Pinaceae of the order Coniferale is one of the most widely used pines of western Himalaya. Its longitudinal and latitudinal distribution ranges extends from 71° to 93°E and 26° to 36°N, respectively. This is a tree of foothills, outer ranges and principal valleys of Himalaya, occuring in a long but comparatively narrow and slightly discontinuous belt reaching from Afghanistan in the West to Bhutan in the East, traversing Northern Pakistan, North-West India and Nepal (Troup, 1921). It usually grows up to 30m in height and 2.5 m in girth, with a cylindrical clean bole of about 12m (Brandis, 1906).

Four, out of the six indigenous Pine species of the Indian subcontinent, viz., Pinus roxburghii Sarg. (chirpine), P. wallichiana A. B. Jacks (blue pine or kail), P. gerardiana Wall. ex Lamb. (chilgoza or nioza pine) and P. kesiya Royle ex Gord. (khasi pine) (P. insularis Endl.), are distributed in the Himalayas. Whereas, P.

armandii Franch., occurs in the North-Eastern provenances and P. merkusii Jhung and de Vriese is found in the hills of Burma (Maheshwari and Konar, 1971). Although four potential resin yielding species of pines are known to occur in India, but Pinus roxburghii is the only species which is commercially tapped for oleoresin.

Knowledge of distribution of variable breeding system of trees and of evolutionary forces that have shaped them is a prerequisite for tree improvement. Such information is also needed in planning provenance testing which is done for very practical reasons to screen the naturally available genetic variation and to allow selection of best available types for reforestation or further breeding programme. The provenance, and/or seed source study also aims at defining the genetic and environmental component of phenotypic variability between trees for different geographic origins. The selection of individual trees on their performance regarding desired traits is useful for interception of variation among trees.

In many parts of the world, considerable work has been done on pine species other than Chir Pine. So there is a need of hour to increase the oleoresin production potential from chirpine trees to narrow down the gap between the requirement and supply. This can be achieved either through horizontal spread of chirpine or vertical improvement of the species through selection of high resin yielding progenies for advanced generation development. But due to increase in population pressure on land there is very less scope of horizontal improvement of oleoresin production, so the only method is the genetic improvement which can be conventionally achieved through selection of high resin yielders among the progenies of plus trees. The present study to test the half sib progenies for oleoresin yield and number of stomata on needles is a step forward in this direction.

Materials And Methods

The present investigation and laboratory analysis was carried out during 2009-2011 under the auspices of the Department of Forest products at Dr.Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan. The oleoresin tapping was done from March to October 2010. The area of investigation is situated at an altitude of 1163-1193 m amsl and lies at 30° 51' N latitude and 77° 09' E longitude. This represents a transitional zone between subtropical and temperate region of the state of Himachal Pradesh. It experiences 1100-1300 mm annual rainfall, most of which occurs during monsoon (June-August).

The study is based on the measurements of trees growing in the genetic trials at the experimental site of the Department of Tree Improvement and Genetic Resources. Open-pollinated seeds of 60 plus trees selected across the entire distribution range of the species in Himachal Pradesh by Dogra (1985) were raised as half-sib families in a replicated progeny trial (Randomised Block Design). The plus trees were selected from 20 Forest Ranges of 12 territorial Forest Divisions of the state of Himachal Pradesh.

Trees of Pinus roxburghii raised in the above mentioned progeny trial were enumerated and abstracted for diameter at breast height (d.b.h). The trees of progenies having more than 30 cm d.b.h were segregated for oleoresin tapping experiment. In total, 22 out of 60 progenies had minimum one tree above 30 cm d.b.h in at least two replications. Hence, these progenies were selected for oleoresin tapping. The method employed for oleoresin collection was bore hole method (Plate 1 and 2) as described by Lekha (2002) and Kumar and Sharma (2005).

For observation and counting of number of stomata on round surface, right flat surface, left flat surface and number of stomata per mm of row, around 15 needles from each tree had been selected randomly. The collected needles were immediately preserved in 100 ml of Formalin, Acetic acid and Alcohol (FAA) solution which consists of 90 ml of 70 per cent ethyl alcohol, 5 ml of glacial acetic acid and 5 ml of formaldehyde (Johansen, 1940). The cross and tangential longitudinal peeling of needles were taken with the help of a sharp blade and were placed in a petridish containing water. These were viewed under the microscope and only the selected sections were used for preparation of permanent slides. The sections were stained as per schedule described by Johansen (1940). The stained sections were mounted on glass slide with the help of DPX mountant (a mixture of Distyrene, a plasticizer and Xylene). Five or six slides of each sample were prepared and best slides were chosen for counting the number of stomata.

The number of stomatal rows on round surface, flat surface 1 and 2 were observed in cross and tangential longitudinal peelings of the needle and counted at 10X magnification in a microscopic field. The

Number of stomata per mm of a row was observed in tangential longitudinal sections of the needle under microscope. The number of stomata was counted at 10X magnification in a microscopic field. The diameter of the microscopic field was measured with the help of stage micrometer which was found to be 1.59 mm. The number of stomata per mm in a row was calculated. Number of stomata per mm of a row was found to be same on both flat surfaces of the needles of a progeny, even then the average of both the surfaces was recorded.

Results and Discussion

The data pertaining to the oleoresin yield from different selected progenies are presented in Table 1. The average value of oleoresin yield was recorded to be 900.51g. The data on number of stomatal rows on round surface (Table 1) exhibited significant variation in different progenies. The photographs of needle sections showing stomatal rows on round surface are shown in Plate 3 and 4. The maximum numbers of stomatal rows on round surface (6.5) were noticed in Leda-P5 and Dibkon-P3. The minimum value (3.0) was recorded in Kaldoo-P10.

The data with respect to number of stomatal rows on flat surfaces of different progenies are appended in Table 1**.** The photographs of needle sections showing number of stomatal rows on flat surfaces are shown in Plate 3 and 5. The effect of all treatments on number of stomatal rows on flat surfaces were found to be non-significant, however, the maximum number was found in kaldoo-P1 and minimum in Kather-PT-Black Centre, Leda-P5 and Rakni-P8.

The perusal to data depicted in Table 1 revealed that the range of number of stomata per mm of a row with in progenies was found to be 6.5 to 12. The photographs of needle sections showing number of stomatal rows on flat surfaces are shown in Plate 4 and 5.

Evaluation of Table 2 revealed that the oleoresin yield had significant and positive correlation with number of stomata per mm of a row (0.410).The non-significant and positive correlation coefficient was observed with Average number of stomatal rows on needle surface.

The data pertaining to genotypic, phenotypic and environmental correlation coefficients are presented in Table 3, at 5 percent level of significance. Oleoresin yield explicated positive and highly significant correlation coefficient at genotypic level, with the number of stomata per mm of a row (0.631). Whereas, rest of the combinations were found to be non-significant.

Data appended in Table 4 depicted high heritability (58.3%) for average number of stomatal rows on needle surface, accompanied by low genetic advance (1.31) and moderately high genetic gain (26.16%). The coefficients of variability were 21.89 per cent and 16.72 per cent at phenotypic and genotypic levels, respectively. Critical examination of data showed very high heritability (68.5%) for number of stomata per mm of a row coupled with low genetic advance (2.45) and high genetic gain (25.43%) for this parameter. The phenotypic coefficient of variability (17.89%) was higher than genotypic coefficient of variability (14.81).

Stomatal density is one common measure of plant response to rising atmospheric Co_2 concentrations (Woodward and Bazzaz, 1988), climate change (Beerling and Chaloner, 1993) and water availability (Beerling et al., 1995). As information continues to increase and new experimental methods for acquiring stomatal densities continue to expand, it is becoming increasingly difficult to compare and interpret stomatal densities reported in literature.

The present investigation reveals that the stomata in Pinus roxburghii are sunken, arranged in rows and found at regular interval as seen in the photograph (Plate 3, 4 and 5). Significant variations are noticed among various progenies for number of stomatal rows on round surface and number of stomata per mm of a row. Whereas, the effect of all treatments on number of stomatal rows on flat surfaces were found to be non-significant. The sunken stomata arranged in rows and found at regular interval is also reported by Rajendra K C (2009) in Pinus merkusii. The results obtained with regard to variability parameters indicate that values have a wide range depicting the presence of good amount of variation existing among the selected progenies. High heritabilities are recorded in number of stomata per mm of a row (68.5%), Average number of stomata on needle surface (58.3%), and oleoresin yield (49.1), which indicate that these parameters are least influenced by the environment. The findings are in agreement with Sagwal and Gupta (1983), Sehgal and Chauhan (1993) and Lekha and Sharma (2006). Oleoresin yield has registered highest values for genetic advance and genetic gain.

Table 1: Variation in oleoresin yield and stomatal density in Pinus roxburghii half-sib progenies

S. No.	Progeny	Oleoresin Yield (g)	Average No. of stomatal rows on		Average No. of stomata per mm of a row
			Round surface	Flat surfaces	
1	Bagthan-PT-Black Centre	1065.0	5.5	2	10.5
2	Bagthan-PT-Black Top	1132.5	5.0	2.5	12.0
3	Banethi-PT-Black Base	852.5	5.5	2.5	8.5
4	Bagthan-PT-Black Base	785.0	5.5	2.5	9.5
5	Chretmansoo-P4	455.0	5.0	2.25	8.0
6	Dhami Shimla Yellow Base	1025.0	3.5	2	10.0
7	Dibkon-P3	685.0	6.5	2	9.0
8	Jainagar-PT-Yellow Base	630.0	3.5	2	10.0
9	Jubble-PT-Green Centre	1425.0	5.5	2.5	10
10	Kaldoo-P1	1112.5	5.0	2.75	9.5
11	Kaldoo-P10	750.0	3.0	2	6.5
12	Kaldoo-P3	930.0	5.5	2.5	8.5
13	Kaldoo-P5	497.5	4.5	2.5	11.0
14	Kaldoo-P8	937.5	4.5	2.5	11.5
15	Kaldoo-P9	700.0	4.0	2	11.5
16	Kather-PT-Black Centre	697.5	5.5	1.75	7.5
17	Kopra-P5	1527.5	6.0	2	11.5
18	Leda-P10	1116.2	3.5	2	10.5
19	Leda-P5	1080.0	6.5	1.75	11.5
20	Leda-P8	980.0	5.0	2	8.5
21	Rakni-P8	667.5	5.5	1.75	7.0
22	Sandrohal-P5	755.0	5.5	3	11.0
Mean		900.5	4.98	2.22	9.70
S.E (d)		226.08	0.70	0.49	0.97
CD0.05		470.16	1.46	N.S	2.02

Table 2: Correlation coefficients between oleoresin yield and other tree parameters.

Character	No. of stomatal rows on round surface	No. of stomata per mm of a row
Yield(g)	0.203	0.410*
Average no. of stomatal rows on needle surface	1.000	0.057
No. of stomata per mm of a row		1.000

*. Correlation is significant at the 5% level (r=0.352)

**. Correlation is significant at the 1% level (r=0.482)

Table 3: Genotypic, phenotypic and environmental correlation between oleoresin yield and other tree parameters

Parameter	Average number of stomatal rows on needle surface	No. of stomata per mm of row
Oleoresin Yield G	0.255	0.631**
P	0.175	0.284
E	0.084	-0.204

*Significant at 5% level of significance (r= 0.352)

**Significant at 1% level of significance (r= 0.482)

Table 4: Estimates of heritability, genetic advance, genetic gain and variability for different parameters of progenies of Pinus roxburghii.

S. No.	Character	Heritability (%)	Genetic advance	Genetic gain (%)	Variability (%)	
					PCV	GCV
1	**Yield**	0.491	320.28	35.51	35.18	24.65
2	**Average number of stomatal rows on needle surface**	0.583	1.31	26.16	21.89	16.72
3	**No. of stomata per mm of a row**	0.685	2.45	25.43	17.89	14.81

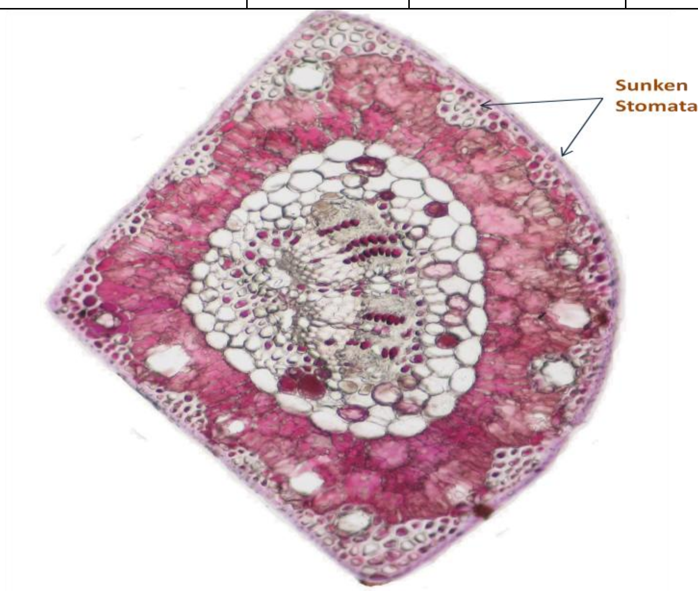

Plate 3: Typical cross section of a Chir Pine needle

Plate 1: Borehole method of oleoresin tapping

Plate 2: Collection of oleoresin

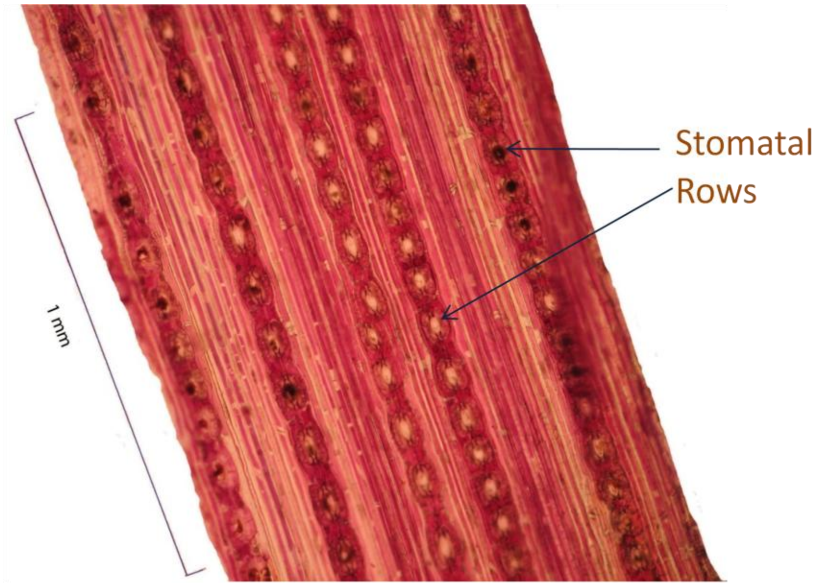

Plate 4: Tangential longitudinal peeling from round surface

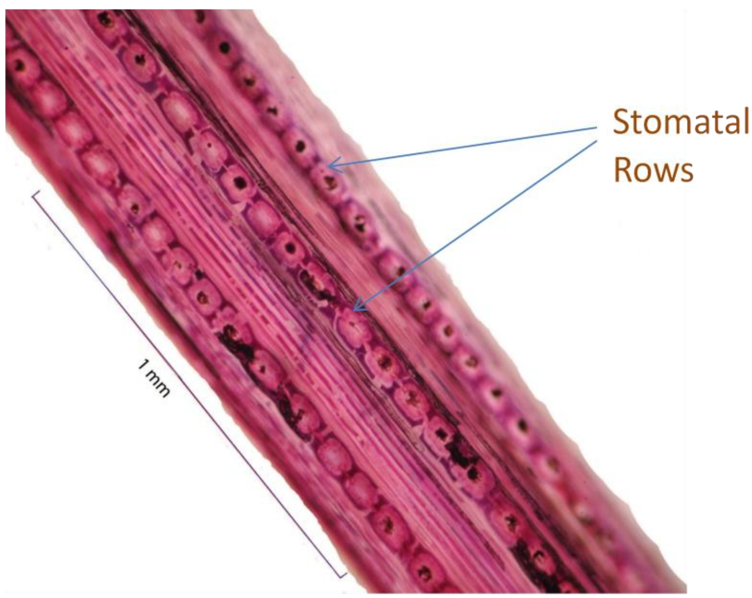

Plate 5: Tangential longitudinal peeling from flat surface

.CONCLUSIONS

Among different progenies of Pinus roxburghii selected for resin tapping, the significant differences have been observed for oleoresin yield, number of stomatal rows on round surface and number of stomata per mm of a row. The maximum value of oleoresin yield has been noted in Kopra-P5 (1527.5 g/season. The lowest oleoresin yield has been found in Chretmansoo-P4 (455.0 g/season. For anatomical traits, Dibkon-P3 and Leda-P5 progenies has recorded maximum number of stomatal rows on round surface (6.5) whereas, Kaldoo-P10 has observed minimum number of stomatal rows on round surface (3.0). Maximum number of stomata per mm of a row (12) has been obtained in Bagthan-PT-Black Top and minimum (6.5) has been observed in Kaldoo-P10. The number of stomata per mm of a row has observed significant relationship with the oleoresin yield. Correlation studies between oleoresin yield and number of stomata per mm of a row projected significant and positive correlation. Genetic correlation coefficient gives an idea about the extent to which the two characters are under the control of the same set of genes or have the same physiological basis of their expression (Jain, 1982). The characters, which are highly correlated, could be directly used for selection. Highly significant and positive genotypic correlation is obtained between oleoresin vs number of stomata per mm of a row.

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