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SEED AND SEEDLING PROVENANCE VARIATION UNDER GREENHOUSE CONDITIONS OF *Pinus caribaea* VAR. *hondurensis* BARR et GOLF [1]

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ABSTRACT - The assessment of 20 provenances of **Pinus caribaea** var. **hondurensis** Barr. et Golf. which represent almost the complete area of natural distribution of the variety, showed that of the nine internal and external seed traits assessed using X-radiography only seed weight and seed length are of value as discriminants for splitting the range into highland and lowland provenances. Average seed weight was 16,5 ± 0,4 mg; Mountain Pine Ridge, Poptun, Potosi and Briones have the heaviest seeds and Piner del Rio, Pantasma and Azacualpa the lightest. Among the seedling traits assessed from germination to one year old under greenhouse conditions, seedling height and root collar diameter after four months show a clinal trend, in which highland provenances have the best growth. The high correlation between seed weight and seedling growth disappears after two months.

RESUMO - A comparação entre 20, procedências de **Pinus caribaea** var. **hondurensis** Barret Golf., as quais representam quase que a totalidade da área de ocorrência natural desta variedade, mostra que das nove características ,internas e externas das sementes, analisadas através de radiografia de raios X, apenas o peso e o comprimento mostraram algum valor como discriminantes entre procedências de diferentes altitudes. O peso médio das sementes foi de 16,5 ± 0,4 mg; as procedências Mountain Pine Ridge, Poptun, Potosi e Briones apresentaram sementes mais pesadas, enquanto que Piner deI Rio, Pantasma e Azacualpa apresentaram sementes mais leves. Entre as características das mudas, desde a germinação até à idade de 1 ano em condições de casa de vegetação, a altura das mudas e o diâmetro do colo aos quatro meses mostraram alguma tendência clinal, sendo o melhor crescimento apresentado pelas procedências de maiores altitudes. A alta correlação entre o peso da semente e o crescimento das mudas desaparece após o segundo mês.

INTRODUCTION

Although the natural distribution of **Pines caribaea** var. **hondurensis** is latitudinally restricted, considerable environmental variation exists among most of the populations. This variety, widely planted in many tropical and sub-tropical countries, has been described by Barret and Golfari (1962); and reviews of the geoclimatic conditions of the natural distribution were given by Lamb (1973) and Greaves (1978).

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[1] The paper is adapted from part of the author's Ph.D. thesis. Forestry Department, Oxford University, England, 1981.

If the expected variation in some seed traits among populations can be associated with the juvenile growth, it will be of great value for early selection or early identification of outstanding provenances or genotypes.

This paper deals with the quantification of the genetic variation of several seed and seedling traits and their interaction throughout the indigenous range of the variety. Significant differences in needle characteristics among natural stands were reported by Salazar (1983). Until now most research has been concentrated on growth traits, production, and genotype-environment interaction (Greaves, 1978; Greaves and Kemp, 1978; Burley and Nikles, 1973) .

Greaves (1980) in an extensive review of the published and unpublished literature on the variety found that, although variation in growth variables has been detected among provenances at the nursery stage, most relevant differences become apparent three or four years after planting. He recognized that the lowland provenances including Karawala, Alamicamba, Brus Lagoon, Poptun, and Santa Clara showed the greatest potential for successful plantation establishment (Fig. 1).

Considerable investigation has been done on seed weight and size and their relation with early seedling growth of species other than **P. caribaea** (Spurr, 1944; Righter, 1945). Kandya (1978) reported those three months after germination, seed weight in **Pinus oocarpa** Schiede as significantly correlated with height, dry weight, and root collar diameter and root length. Kandya also suggested that seed weight can be used for predicting seedling growth.

Variation in seed quality occurs as a result of climatic conditions, crown position and crown orientation; this variation normally is reflected in early seedling variation (Kamra and Simak, 1968; Basada, 1979).

Working under greenhouse conditions with **P. caribaea** var. **hondurensis**, **P. caribaea** var. **bahamensis** and **P. caribaea** var. **caribaea** Jara (1979) found significant differences in almost all the seed traits and in most of the seedling traits assessed. However, a clear separation between populations could be made only by seed weight where Mountain Pine Ridge, Potosi and Los Limones showed the heaviest seeds, and Alamicamba the lightest. In this case latitude and length of the dry season appeared to be important in seed morphology; and seed weight, seed width, cotyledon length and number of cotyledons largely accounted for the total variability of the seeds and seedling growth.

X-ray seed analysis-which is simple, accurate, quick and repeatable, is useful tool in seed studies, particularly to relate some internal seed traits with seedling behavior (Kamra, 1972).

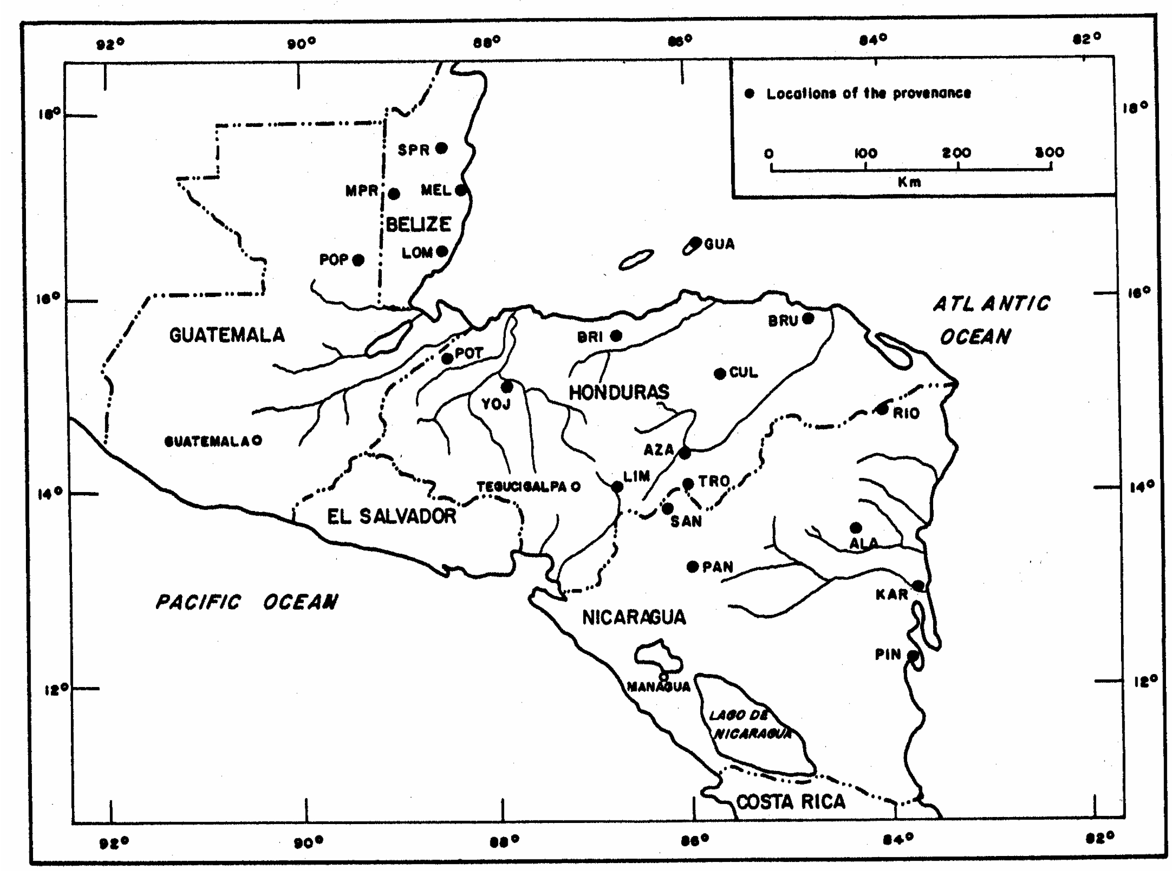

Figure 1. Map of Central America showing provenance locations of P. caribaea var. hondurensis assessed for seed and seedling traits

MATERIALS AND METHODS

The seeds assessed in this study were collected by the Commonwealth Forestry Institute (CFI) staff of Oxford University, England, between 1970 and 1978, and stored at 3 ± 1°C and about 8% moisture content. Detailed information of the provenance location and seed collection is given by Greave (1978). Details of the 20 seed sources analyzed are given in Table 1 and Figure 1.

The X-ray techniques outlined by Kamra (1972), were performed to assess most of the seed traits. Eighty seeds of each provenance, mounted on double transparent sellotape stuck on sheets of Industrex (registered mark of Kodak film) C film were radiographed with soft X-ray, using 17KV, 10 mA, 2,5 m local length and 4 minutes of exposure. The combination of these factors gave a dose of 0,75 rem, which .is considered very low.

Based on the radiographies, 30 full seeds with embryos and without mechanical damage were selected per provenance. The radiograph of each seed selected was examined with a Projectina projection microscope at 10x magnification, and the following traits recorded:

Seed weight (sw)
Seed length (sl)
Seed breadth (sb)

Seed coat thickness (sct)
Endosperm length (el)
Endosperm breadth (eb)
Embryo breadth (emb)
Embryo length (eml)
Embryo cavity occupation (eco)

Seed weight was recorded to the nearest milligram, and the other traits to the nearest millimeter. Seed length was measured over the seed coat along the longest axis of the seed; seed breadth was assessed on one of the widest faces at the middle of the seed; seed coat thickness was recorded at the center; endosperm length and breadth were recorded in a manner similar to that described for seed length and breadth; embryo breadth was assessed at the union of the suspensor; embryo length was measured as the distance between the suspensor and the tip of the cotyledons; embryo cavity occupation, the area of the cavity occupied by the embryo, was classified as fully, three-fourths or one-half occupied. Each selected seed was labelled and stored in a separate bag until sown. The seeds were sown in the greenhouse at Wytham Field Station, Oxford University, in an inoculated mixture of sand and peat (1:1). The seedlings were grown for 12 months under partially controlled temperature and relative humidity. The mean temperature over the year was 21 ± 3°C, and relative humidity was approximately 45% during the day and 75% at night. The light followed the natural and seasonal intensity of the region. After germination the seedlings were hand watered every three or four days, and fertilized every two months with 75 cc per pot of 50 cc of a commercial liquid fertilizer (Maxicrop International Ltd. ,England) diluted in 4,5 1 of tap water.

Table 1. Source and climatic variables for 20 provenances of Pinus caribaea var hondurensis assessed for seed and seedling traits.

Country	Origin	Code	Latitude (N°)	Longitude (° W)	Altitude (masl)	Rainfall (mm)	Dry Months	Mean temperature (°C)
Nicaragua	Pinar	(PIN)	12° 13'	83° 42'	10	4187	1	26,4
Nicaragua	Karawala	(KAR)	12° 58'	83° 43'	10	3897	0	26,4
Nicaragua	Pantasma*	(PAN)	13° 20'	85° 57'	475	1400	5	20,7
Nicaragua	Alamicamba	(ALA)	13° 34'	84° 17'	25	2610	3	27,3
Nicaragua	Santa Clara*	(STA)	13° 48'	86° 12'	700	1818	5	23,4
Honduras	Trojes*	(TRO)	14° 03'	85° 58'	720	1649	5	23,0
Honduras	Los Limones*	(LIM)	14° 03'	86° 42'	700	663	7	22,2
Honduras	Azacualpa	(AZA)	14° 25'	86° 07'	240	2131	3	25,7
Nicaragua	Rio Coco	(RIO)	14° 45'	83° 55'	75	2863	2	30,4
Honduras	Yojoa*	(YOJ)	14° 58'	87° 54'	600	2995	2	24,0
Honduras	Culmi*	(CUL)	15° 06'	85° 37'	550	1325	6	24,3
Honduras	Potosi*	(POT)	15° 20'	88° 25'	650	1205	7	23,7
Honduras	Los Briones*	(BRI)	15° 34'	86° 44'	600	912	6	24,0
Honduras	Brus Lagoon	(BRU)	15° 45'	84° 40'	10	2840	2	26,5
Guatemala	Poptun	(POP)	16° 21'	89° 25'	500	1688	4	24,2
Honduras	Guanaja Island	(GUA)	16° 27'	85° 54'	75	2308	3	27,1
Belize	Los Lomitas	(LOM)	16° 28'	88° 33'	30	2398	3	27,1
Belize	Mountain Pine Ridge*	(MPR)	16° 58'	89° 00'	487	1558	3	23,9
Belize	Melinda	(MEL)	17° 01'	88° 20'	12	2137	2	26,9
Belize	Santos Pine Ridge	(SAN)	17° 34'	88° 33'	30	1818	2	26,2

The experimental design was a randomized complete block with 20 seeds sources, three replications, and five trees per plot in individual pots. Two seeds were sown in each pot to assure the presence of 15 seedlings per provenance; excess seedlings were discarded. In this way seed and seedling identity were maintained throughout.

The following twenty-three traits were recorded following germination:

Hypocotyl length (cm)	(hil)
Cotyledon number	(cn)
Cotyledon length (cm)	(cl)
Height at 2 months (cm)	(ht2)
Number of branches at 2 months	(br2)
Height at 4 months (cm)	(ht4)
Number of branches at 4 months	(br4)
Secondary needles at 4 months	(sn4)
Height at 6 months (cm)	(ht9)
Diameter at 6 months (mm)	(di6)
Number of branches at 6 months	(br6)
Secondary needles at 6 months	(sn6)
Length primary needles at 6 months	(1pn)
Height at 8 months (cm)	(ht8)

Number of branches at 8 months	(br8)
Secondary needles at 8 months	(sn8)
Height at 10 months (cm)	(ht 10)
Number of branches at 10 months	(br10)
Secondary needles at 10 months	(sn10)
Height at 12 months (cm)	(mm)
Diameter at 12 months (mm)	(di12)
Number of branches at 12 months	(br12)
Secondary needles at 12 months	(sn12)

RESULTS AND DISCUSSION

Significant differences were found between provenances for all seed traits measured, with the exception of seed coat thickness (Table 2). Weight and length of the seed showed 26,4% and 18,5% variation among provenances, respectively. The variation for the other traits ranged between 1,1% and 14,33%, which is very low, especially when the sensitivity of the seeds to environmental conditions and the collection of the seed in different years (up to eight years difference) are taken into consideration.

The average seed weight was 16,5 ± 0,4 mg; high latitude and altitude provenances - Mountain Pine Ridge, Poptun, Potosi, Briones and Los Limones - showed 80%, 73%,60% and 40% respectively of the seeds with weight over 15 mg. This trend is supported by the multiple range test (Figure 2), where these populations were ranked highest. An exception was the provenance Pantasma, which had light seed. Highland populations are subject to a dry period of three to seven months and receive an annual rainfall ranging from 663 to 1 800 mm; their longer seed means more water and nutrients can be stored, and this might be interpreted as na adaptation to dry conditions.

The trend for a thicker seed coat in the highland provenances could be an adaptation to the dry-season climate.

A grouping tendency of seed weight, seed length, endosperm length and embryo length for most of the provenance with respect to altitude can be observed in Fig. 3. The association is especially strong for seed weight, with the exceptions of Pantasma and Azacualpa from high altitudes, which show ecotypic variation.

Of the 23 traits assessed on seedlings to twelve months old, hypocotyl length, cotyledon length, height and diameter growth at all measurements were significantly different among the 20 provenances. Between-provenance variation ranged from 0,30% to 21,8%. More than 62% of the total variation detected in all the 23 traits assessed was explainable as variation between seedlings within each provenance (Table 3).

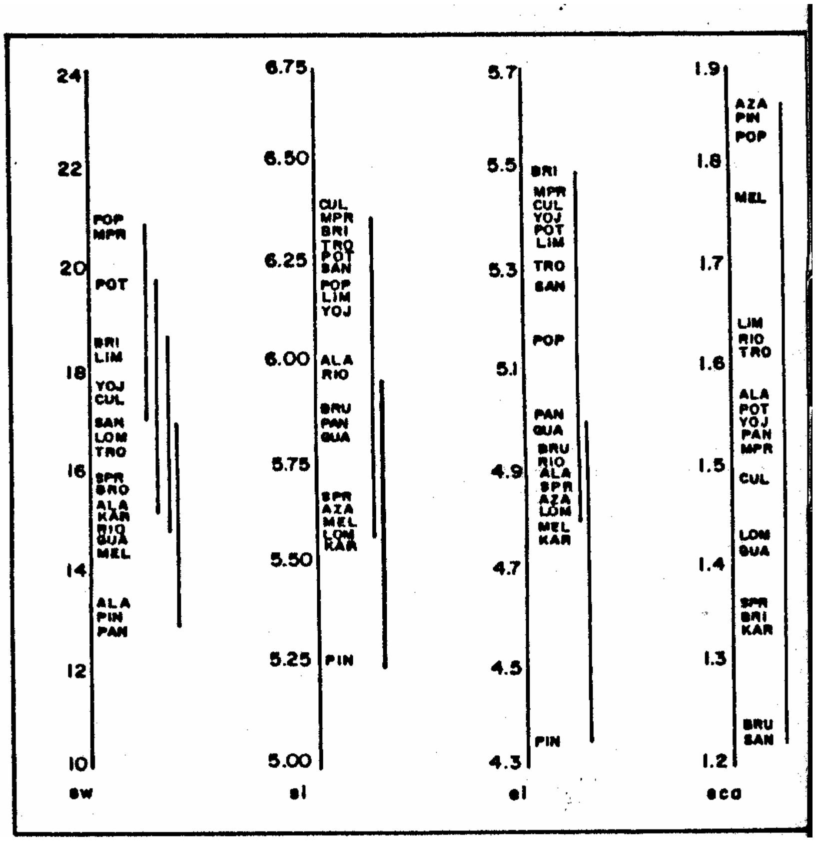

Figure 2. Studentized range test for seed traits (p < 0.05) of 20 provenances of P. caribaea var. hondurensis

Table 2. Significance of F-rations and variance component percentage for seed traits

Entry Nº	Sources of variation	SEED TRAIT								
		sw	**sl**	**sb**	**sct**	**el**	**eb**	**emb**	**enl**	**eco**
1	Provenance (P)	***	***	**	NS	***	*	**	***	*
2	Replications (R)	NS	NS	NS	NS	NS	NS	NS	NS	NS
3	P x R	NS	NS	NS	NS	NS	NS	NS	NS	NS
		VARIANCE COMPONENTS (%)								
1	P	26,41	18,53	10,68	1,13	14,32	10,40	5,37	12,52	5,52
2	R	0,34	0,00	0,61	0,08	0,00	0,81	0,62	0,00	0,10
3	P X P	0,00	0,00	4,68	1,11	0,16	6,05	0,00	0,00	0,00
4	Seeds in R in P	73,25	81,47	84,63	97,69	85,52	82,74	94,02	87,48	94,38

* significant at P < 0,05 of probability
** significant at P < 0,01 of probability
*** significant at P < 0,001 of probability
NS not significant at P < 0,05 of probability

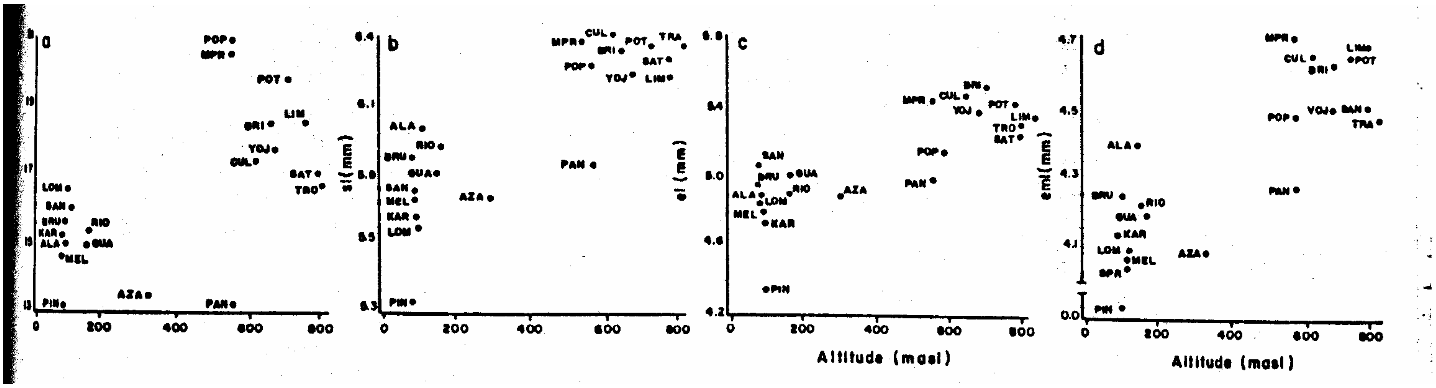

Fig 3. Relationship between (masl) and: a) seed weight (sw); b) seed length (sl); c) embryo length (eml) of 20 provenances of P. caribaea var. hondurensis.

Table 3. Summary of analyses of variance and variance components for seedling traits

Entry sources of variation	SEEDLING TRAIT																						
	hil	**cn**	**cl**	**ht2**	**br2**	**ht4**	**br4**	**sn4**	**ht6**	**di6**	**br6**	**sn6**	**lpn**	**ht8**	**br8**	**sn8**	**ht10**	**br10**	**sn10**	**ht12**	**di12**	**br12**	**sn12**
	F - RATIO																						
1 Provenance (P)	***	NS	***	**	NS	***	NS	NS	***	**	NS	NS	NS	***	NS	NS	**	NS	NS	***	**	NS	NS
2 Replications (R)	*	NS	NS	**	NS	**	NS	NS	***	NS	NS	*	NS	***	NS	***	***	NS	**	***	NS	NS	**
3 P x R	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	*	NS	*
	VARIANCE COMPONENTS (%)																						
1 P	13,33	5,38	19,23	11,32	2,04	21,81	0,96	4,61	16,48	11,85	0,72	2,33	4,10	18,74	1,06	1,18	13,34	0,30	9,71	14,48	14,28	1,05	5,33
2 R	2,66	0,00	1,31	7,88	1,41	6,52	0,00	0,78	8,57	1,34	0,00	3,90	0,00	9,49	0,00	1,31	15,71	0,00	12,93	12,93	12,48	0,00	7,61
3 P x R	0,00	1,18	0,00	5,75	0,00	3,00	0,34	5,76	6,15	3,09	0,00	5,77	2,19	4,52	0,00	5,52	4,50	0,00	6,93	3,13	7,58	0,00	9,13
4 Seeds in R in P	84,01	93,43	79,46	75,04	96,55	62,67	98,00	88,85	68,80	83,72	99,28	88,00	93,71	67,25	98,95	81,94	66,46	99,70	70,43	69,91	78,14	98,95	77,93

* significant at P < 0,05 of probability
** significant at P < 0,01 of probability
*** significant at P < 0,001 of probability
NS not significant at P< 0,05 of probability

All the highland provenances plus the Guanaja Island population have longer hypocotyls and cotyledons than the overall provenance means (2,85 ± 0,18 cm and 2,84 ± 0,14 cm respectively). The multiple range tests (Figure 4) show that the small differences do not allow a clear separation between.

The effect of seed weight on seedling growth was evident or11y up to two months of age, principally for highland provenances; the exception was for provenances with the smallest seeds such as Alamicamba and Pantasma (Figura 5 and 6). Beyond age two months no relationship was found between height growth and the seed traits. With the exceptions of Mountain Pine Ridge, Santa Clara, Alamicamba, and Los Limones, which showed a clear growth-age interaction, remaining provenances displayed consistent relative growth patterns beyond age four months. Lomitas from lowland and Potosi from highland were consistently superior in growth; Pinar showed the poorest growth. Because of this inconsistency during the first four months of seedling growth, the trait must be used with caution to predict later growth. Growth during the latter eight months was more consistent; nevertheless more research is needed to determine long-term trends.

Univariate analysis suggests a clinal tendency in height growth up two months age, which could be an effect of seed size; after four months the tendency is more ecotypic. Although seedling growth is frequently used to evaluate vigour, it must be used with care, since vigour in height and seed traits can be influenced by a large number of environmental variables (Nanda et al., 1969).

At 6 and 12 months there was evidence of ecotypic variation in root collar diameter among provenances. Although at 12 months the multiple range test did not give a clear separation of the provenances, Trojes, Santa Clara and Brus Lagoon, which had the longest root collar diameters, could be considered to be different populations from the rest; a similar approach could be taken with Pinar population (smallest diameter and total height) .

There was no relationship between height root collar and diameter at 12 months- possibly an effect of high density in the greenhouse limiting the crown growth and therefore stem diameter growth.

Cotyledon numbers, branch numbers and needle numbers were not significantly different among provenances. Hypocotyl length, cotyledon number, cotyledon length and height at two months showed a similar behaviour to that reported by Jara (1979); the lack of systematic variation in the other traits can be interpreted as the result of genotype-environment interaction at this juvenile stage.

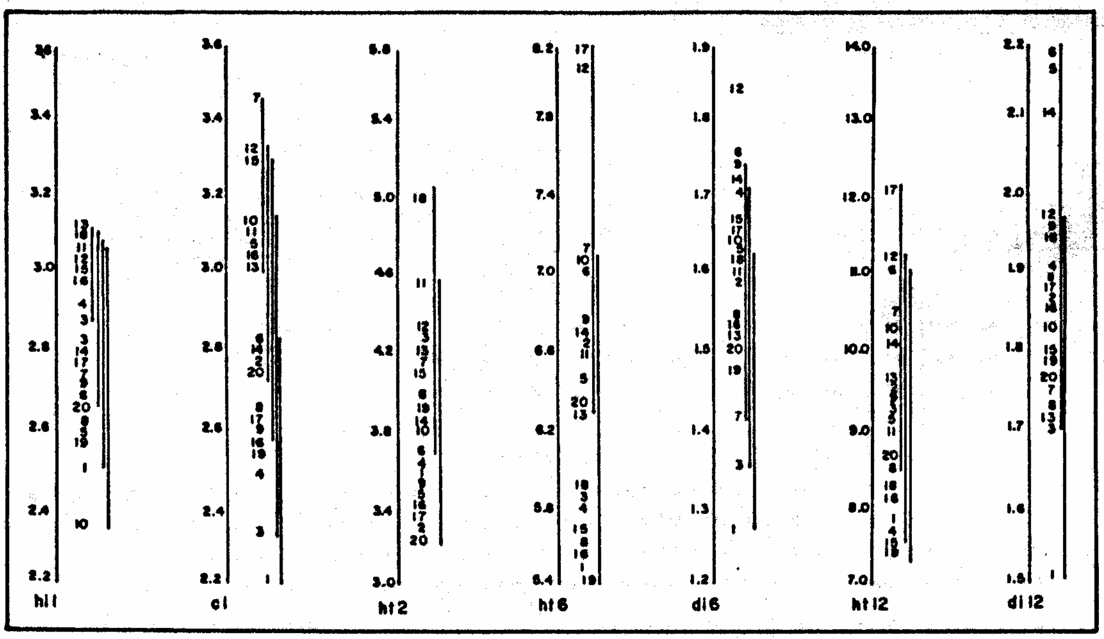

Figure 4. Studentized range test for some of the seedling ($p < 0.05$) of 20 provenances of P. caribaea var hondurensis

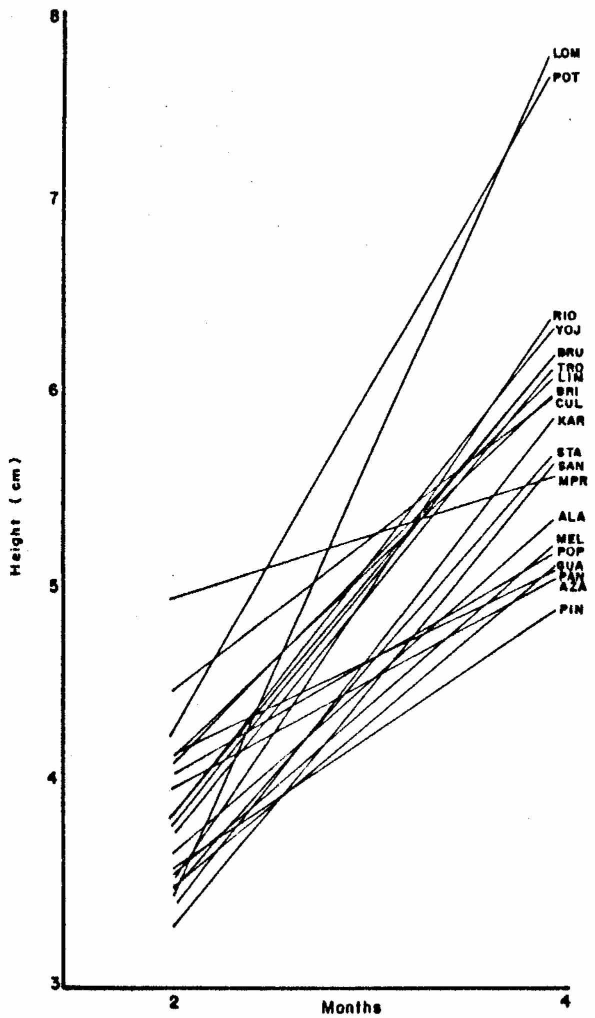

Figure 5. Height growth of 20 provenances of P. caribaea var. hondurensis at two and four monthes age.

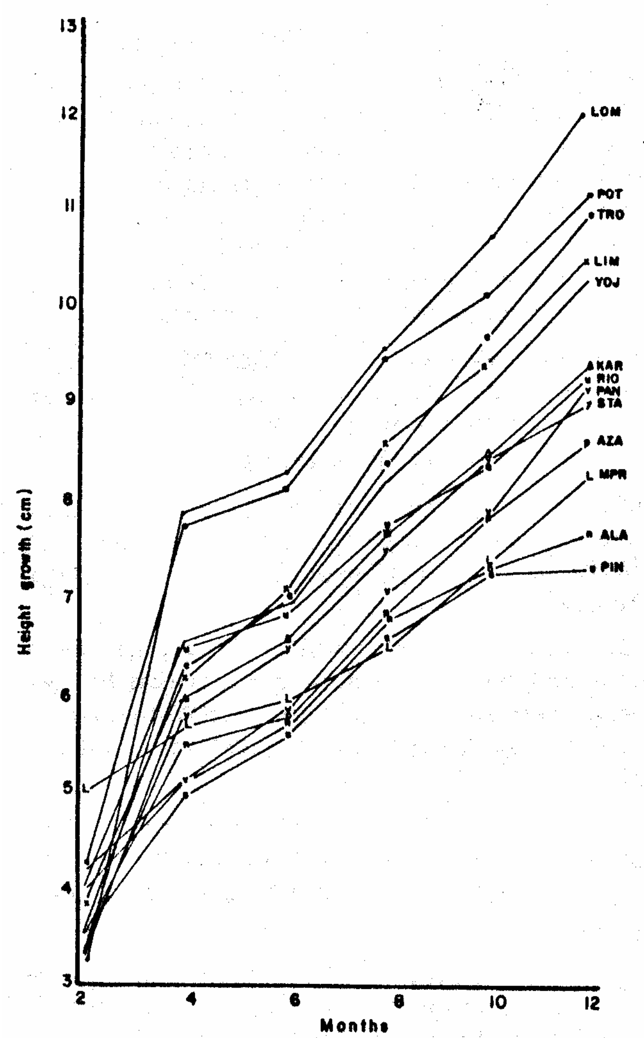

Figure 6. Height growth of 13 provenances of P. caribaea var. hondurensis during 12 months growth.

The late secondary needle development in highland populations could be a result of low transpiration of the seedlings. However, Jara (1979) found more needles at eight months in highland populations, with the exception of Los Limones.

The poor seedling growth of the third group could be associated with poor genotypes as a result of differentiation of superior stock through commercial exploitation (Greaves, 1978). In those populations a high proportion of trees exhibit poor form, while the scattered isolated Populations increase the possibility of decline due to in-breeding or crossing with parents of poor genetic quality.

CONCLUSIONS

Al though the amount of variation in seed traits between provenances is relatively low, it was generally possible to detect a clear trend with respect to altitude. The longest and heaviest seed means are those of the highland populations. This may be the result of adaptation to lower rainfall and the need to store more water and nutrient to ensure seedling survival under the harder upland environmental conditions.

Seed weight was found to be the seed trait best correlated with early seedling growth. Longer seeds with large food reserves and large embryos produce longer hypocotyls and shoots at two months. After this age seedling development under greenhouse conditions appears to be unaffected by seed traits .There is no reason to select bigger seeds, apart from the nursery advantage of bigger seedlings. Similar results were reported by Venator (1976) from 12 of the same provenance used in this study.

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