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Changes in the hormonal balance during seedling growth under salt stress in pigeon pea (*Cajanus cajan*, L.).

Resumen. Se estudió la variación del nivel de reguladores del crecimiento en los primeros estadíos de crecimiento de plántulas de *C. cajan*. bajo concentraciones isotónicas de Na Cl y Na_2SO_4. Ambas sales redujeron inicialmente el nivel de los promotores del crecimiento, con un aumento simultáneo en inhibidores del tipo ABA. Posteriormente se observó un incremento gradual en sustancias del tipo de la giberalina y la citoquinina y una reducción en sustancias del tipo ABA con el crecimiento de las plántulas. las cuales mostraron una recuperación en los períodos posteriores. Sin embargo, la inhibición se perpetuó durante todo el estudio. con un efecto más marcado del Na_2SO_4 que del Na Cl.

Salinity affect plant growth and influence several facets of plant metabolism. Several reports indicate changes in the levels of endogenous growth regulators under stress conditions. Ramana (11) observed measurable reduction in gibberellin activity under saline conditions in groundnut, and a reduced cytokinin activity in root exudate was observed when plants were subjected to water deficits (4) or to salt stress (5,9). Increased levels of ABA were reported to accumulate under water stress (8). Most of the earlier studies indicate that changes in hormonal balance during saline conditions depend more on the total concentration of soluble salts than on specific ions (7). Nevertheless this by no means rules out the possibility of a direct effect of salt on hormone levels (6).

The effect of salinity on plants varies depending on the phase of development. There is a lack of general relationship between the relative salt effect on germination and later phases of seedling growth. Studies on the influence of specific ions on the hormonal balance during a period of growth are scanty. Hence the present investigation is designed to study the effect of isotonic concentrations of NaCl and Na_2SO_4 salts on initial and subsequent changes in the endogenous levels of growth regulators during early seedling growth in pigeon pea (*Cajanus cajan* L.).

Material and methods

Plan Material: Healthy seeds of pigeon pea (*Cajanus cajan* L.) were surface sterilized with 0.1% $HgCl_2$ for 2-3 min and thoroughly washed with distilled water. Petridishes containing the seeds were lined with filterpaper and were divided into three sets. The first two sets were separately treated with iso-tonic solutions of NaCl and Na_2SO_4 (2.9 atmospheric pressure) and the third set which served as control, received distilled water. Five replicate samples were maintained for each treatment. Fifty germinated seeds were allowed to grow at 29 + 2°C in each petridish. The salt solutions were renewed daily to prevent evaporation losses and contamination. The endogenous levels of gibberellin and cytokinin-like substances and ABA-like inhibitors and growth patterns relating to fresh and dry weights and root and shoot lengths were determined in zero-, 3-, 6-, and 9- day old seedlings.

Extraction and purification

Growth regulators were extracted and chromatographed according to the method of Rudnicki and Nowak (14). Fifty grams of plant material (whole of seedling) were homogenized in 200 ml of 80% (v/v) methanol. The water residue was acidified to pH 2.5 and extracted with ethyl acetate. The combined ethyl acetate extracts were washed with 4% (w/v) sodium bicarbonate. The bicarbonate phase was readjusted to pH 2.5 and again extracted with ethyl acetate. The ethyl acetate fraction was evaporated until dry and dissolved in methanol. Ten grams equivalent of this final sample were line-loaded on Whatman No. 1 chromatography paper.

The cytokinin containing aqueous phase was evaporated to volume of 20 ml and passed through a Dowex 50 W-X-4H (50-100 mesh) and eluted with 2N aqueous ammonia. The ammonia was evaporated under vacuum to a small volume and line-loaded on Whatman No. 1 paper.

Chromatography and biossay

The dry residues of ethyl acetate and ammonia fractions on the chromatographic paper were developed in a mixture of isopropanol:ammonia:water (10:1:1 by vol.). They were then airdried and cut into 10 equal R_f zones and bioassayed for determination of growth regulators.

The biological activity of gibberellins was evaluated on the basis of cucumber hypocotyl elongation bioassay (12). The presence of cytokinin activity was determined with cucumber cotyledon greening bioassay (3). Wheat coleoptile straight growth bioassay was carried out for ABA-like inhibitors (13).

Results

As shown in Table 1, the linear growth and fresh and dry weights of the seedlings decreased markedly in both salt treatments, but the effect of Na_2SO_4 was slightly more pronounced than NaCl. Shoot length rather than the root length was more affected by salt treatments. Relatively slow pace of growth and more water accumulation was observed in treated plants during the initial stages of growth.

Changes in the growth regulators

In the control plants more gibberellin activity was observed at R_f 0.2-0.3, 0.4-0.5, 0.6-0.7 and 0.9-1.0 at all the stages of plant growth, whereas treated plants differed to some extent (Figure 1). Cytokinin-like substances showed maximum activity at R_f 0.3-0.4 and 0.7-0.8 in both control and treated plants (Figure 2). ABA-like inhibitor activity was intense at R_f 0.2-0.4, and 0.7-0.9 (Figure 3) in both control and treated plants.

Gibberellin and cytokinin-like substances showed considerable reduction in NaCl treated plants than that of control ones, at all stages of growth. Similar and even more pronounced results were obtained with Na_2SO_4 treated plants. A substantial increase in the activity of ABA-like substances was observed in both the treatments over the control. Here also Na_2SO_4 treated plants were showing a little more inhibitory activity. Though the effect was similar, the difference between the two salts was significant.

There was a linear increase in both gibberellins and cytokinins from the third to the ninth day with an increase in growth in both control and treated plants (Figures 1 and 2), while ABA-like inhibitors showed gradual reduction during this period in all the cases (Figure 3). This trend was significant from the sixth to the ninth day, where the seedlings showed a slight recovery in the physical growth from the stress.

Discussion

From the foregoing results it is inferred that during salt stress the endogenous levels of gibberellin and cytokinin-like substances were acutely affected with a concomitant increase in ABA-like inhibitors.

Table 1. Effect of iso-osmotic concentrations of NaCl and Na_2SO_4 on seedling growth of *Cajanus cajan* L. Values are mean ± S.D.

Observation	Seedling age in days								
	Control			NaCl			Na_2SO_4		
	3	6	9	3	6	9	3	6	9
Shoot length (cm)	2.01 ±0.146	9.62 ±0.802	18.14 ±1.421	1.53 ±0.117	4.76 ±0.546	10.92 ±0.826	1.46 ±0.127	4.06 ±0.394	9.31 ±0.681
Root length (cm)	2.74 ±0.191	7.41 ±0.529	9.84 ±0.584	2.27 ±0.218	5.84 ±0.620	8.08 ±0.612	2.08 ±0.124	5.22 ±0.534	7.66 ±0.711
Root/Shoot ratio	1.39	0.78	0.54	1.49	1.24	0.74	1.43	1.28	0.89
Fresh weight (mg)	105.2 ±2.834	276.6 ±7.426	355.0 ±9.086	126.0 ±2.991	243.3 ±8.024	316.0 ±9.621	135.0 ±4.023	224.0 ±6.692	305.1 ±8.721
Dry weight (mg)	22.3 ±1.011	58.1 ±2.012	71.0 ±3.042	17.6 ±1.926	36.0 ±2.128	62.2 ±3.109	16.3 ±1.762	32.4 ±2.431	60.3 ±2.807

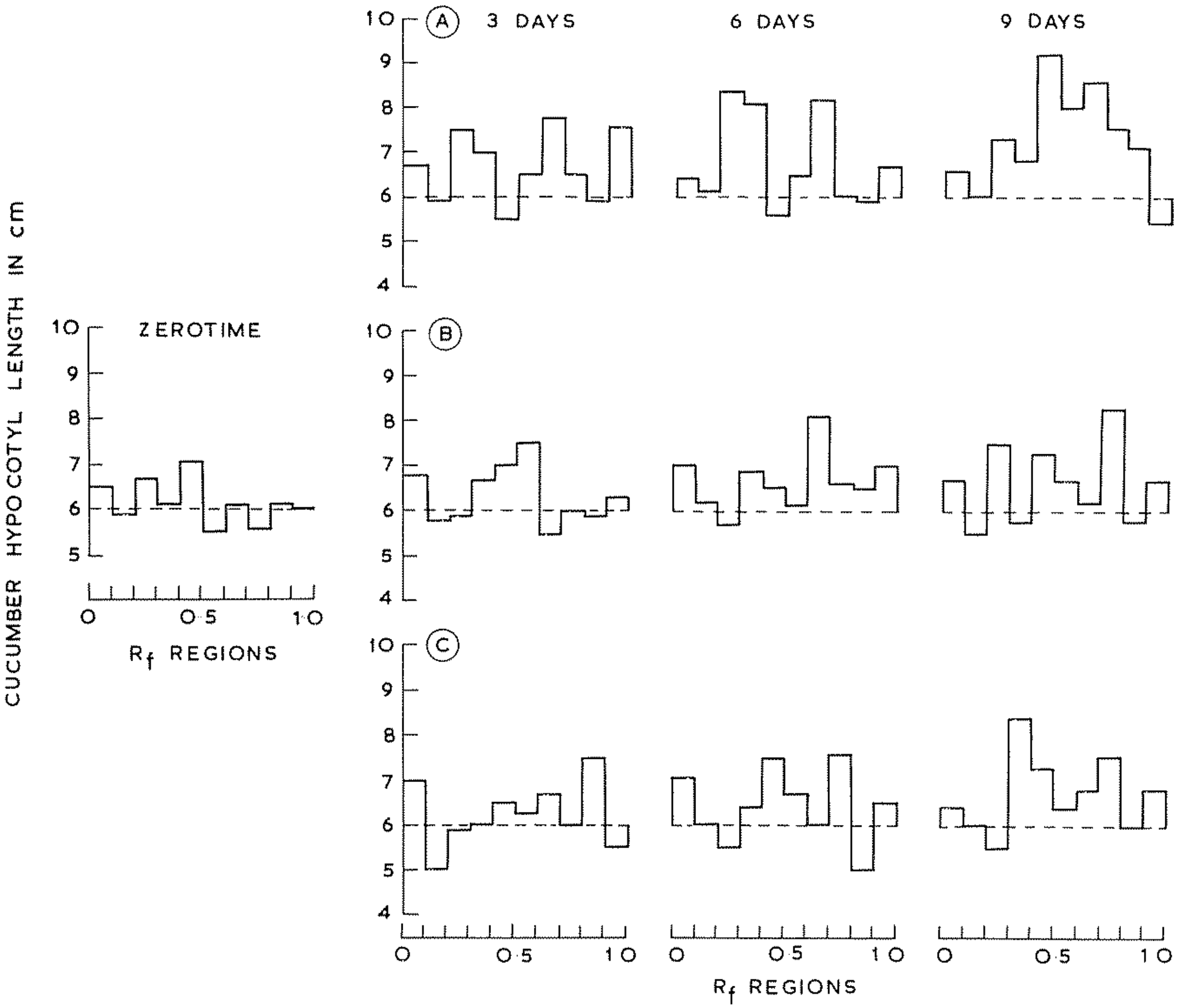

Fig. 1. Changes in the activity of gibberellins in pigeon pea seedlings, determined with cucumber hypocotyl elongation bioassay. Histograms represent (A) Control, (B) NaCl treated and (C) Na_2SO_4 treated. The values are mean of three separate bioassays of 50 g each.

The variations in growth, fresh and dry weights and in the levels of endogenous growth regulators exerted by NaCl and Na_2SO_4 at the osmotic pressure of 2.9 atm might indicate the ionic effect of these salts besides the osmotic effect, in which case Na_2SO_4 seems to be more toxic than NaCl. The gradual and slight increase of growth promoters and decrease in ABA-like inhibitors of the treated plants with the progressive growth, are in confirmity with the growth patterns which showed a slow recovery of the plant from the initial stress.

The presence of higher levels of cytokinin-like substances with reduced ABA-like inhibitor accumulation in the control plants supports the view of Zee-Even and Itai (15), that cytokinins effect ABA inactivation by increasing bound ABA levels, finally resulting in enhancement of plant growth. In the salt treated plants, the decreased cytokinin levels and increased ABA levels suggest the probability that ABA accumulation may be responsible for lower cytokinin activity during the stress, by inhibiting either byosynthesis or activity of cytokinins (1,2). The changes in gibberellin activity might well relate to the presence of inhibitor content (10). The inactivation may be a result of inhibitor accumulation during stress, and ABA is one of these inhibitors under such conditions.

The accumulation of ABA-like inhibitors and low levels of growth promoters in the early seedling stage and their progressive reversion in the latter stages

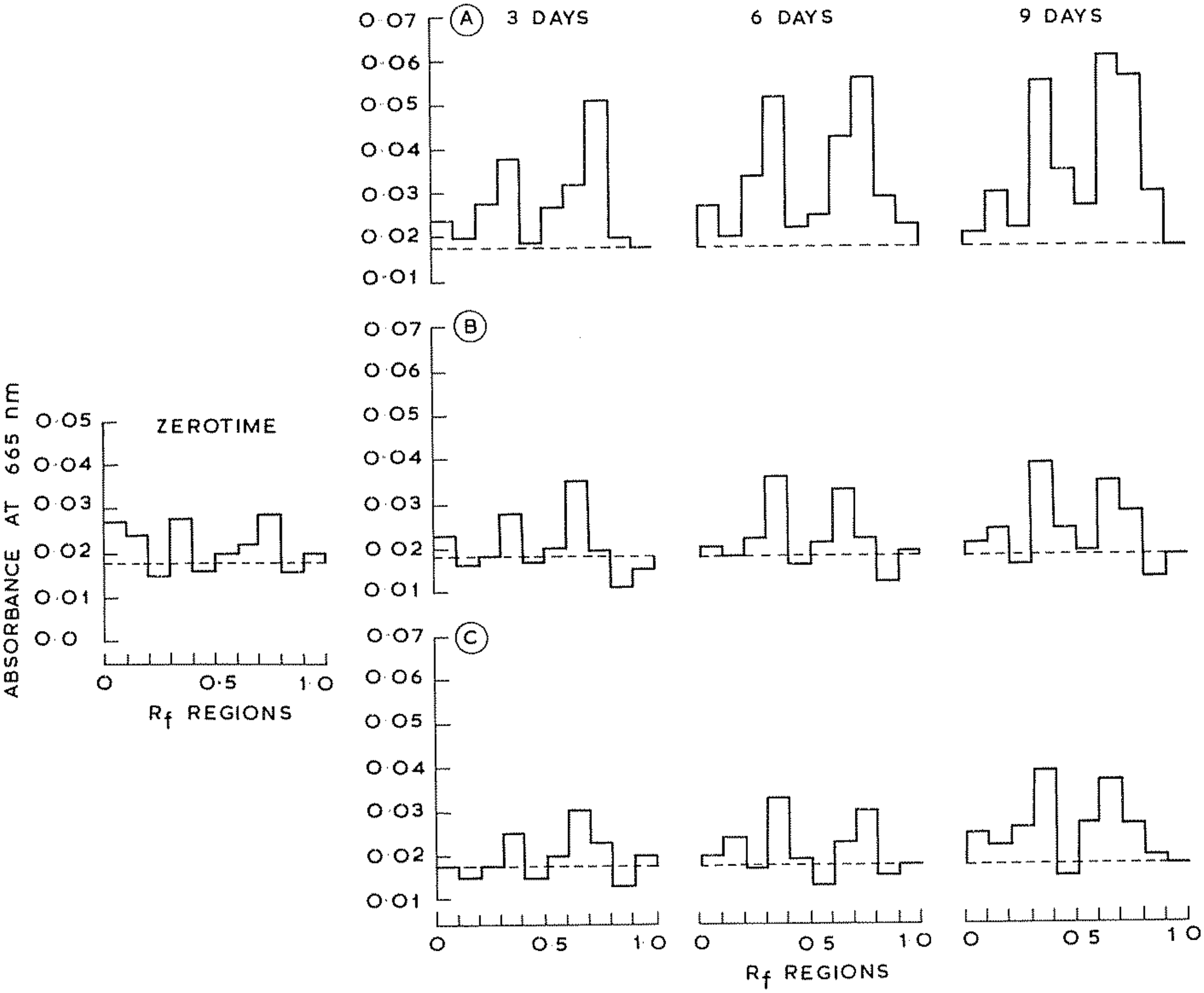

Fig. 2 Activity of cytokinins in pigeon pea seedlings of control (A) NaCl treated (B) and Na_2SO_4 treated (C), determined with cucumber cotyledon greening bioassay. The values are mean of three separate bioassays of 50 g each.

show the sensitivity of the plant during germination. This suggests a more complex mode of interaction among growth hormones during stress condition. In view of these results this phenomenon may be interpreted as a reflecting feature of the regulatory mechanism that facilitates the plants an adaptive response to a decreased osmotic potential of the root medium and a recovery thereafter in its progressive growth.

Summary

The levels of endogenous growth regulators in pigeon pea during early seedling growth under isotonic concentrations of NaCl and Na_2SO_4 were studied. Both NaCl and Na_2SO_4 initially reduced the levels of growth promoters, with a simultaneous increase in ABA-like inhibitors. But a subsequent substances and a decrease in ABA-like inhibitors was observed with the progressive growth of the seedlings, which showed a slow recovery in the later stages. However, the inhibition was significant in stages. However, the inhibition was significant in treated plants throughout the growth period, where Na_2SO_4 was found to be more effective than NaCl.

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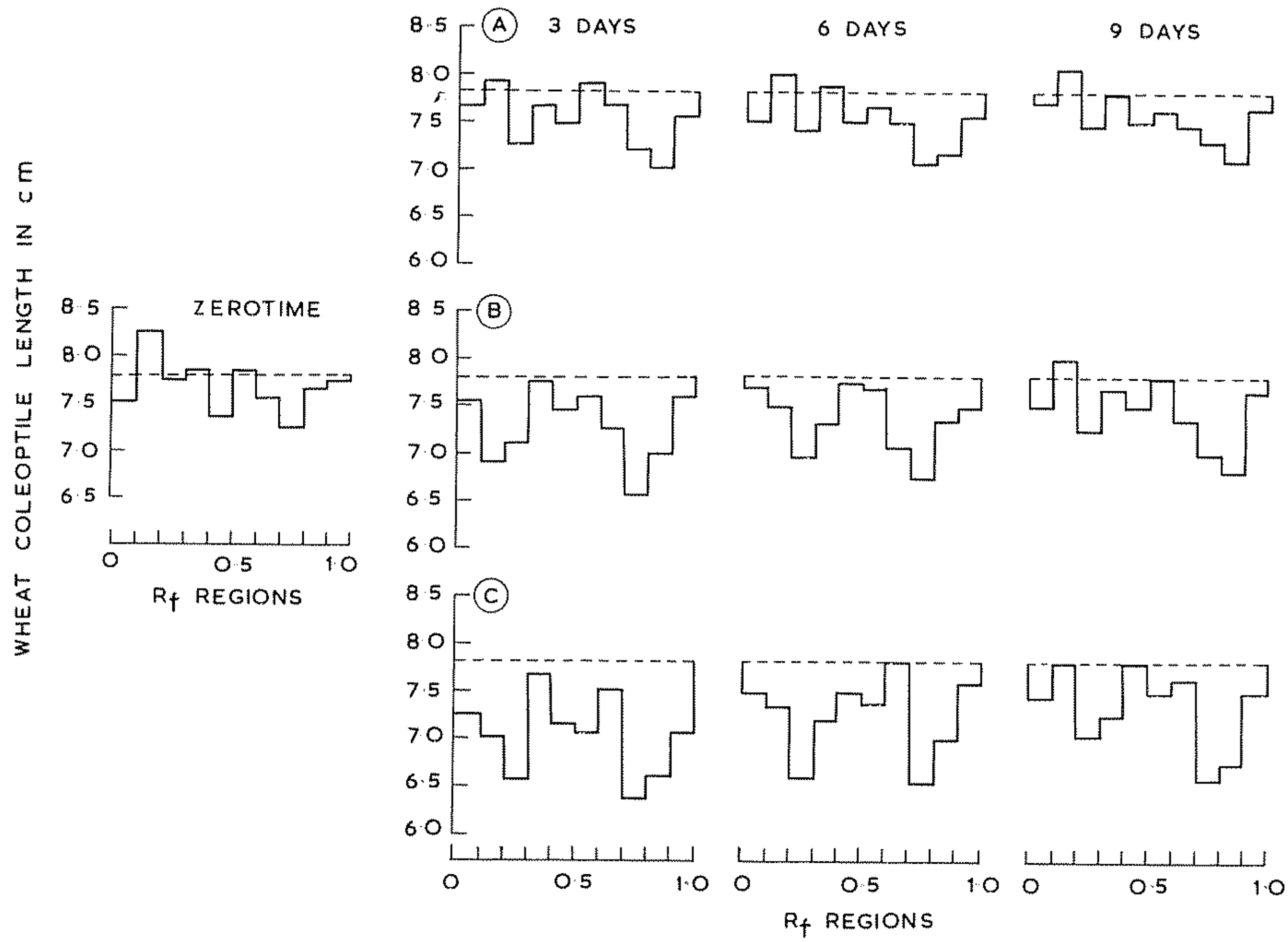

Fig. 3. Changes in the activity of ABA like inhibitors in pigeon pea seedlings of control (A) NaCl treated (B) and Na_2SO_4 treated (C). determined with wheat coleoptile straight growth biossay The values are mean of three separate bioassays of 50 g each

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Aluminio activo en suelos derivados de cenizas volcánicas de Costa Rica y Guatemala.

Summary Active aluminum extracted with 0.2 M ammonium oxalate, pH 3.0, and 4 M KOH of 51 samples of Andepts of Costa Rica and Guatemala was quantified and compared. Acid-oxalate extractable alumimum varied from 0.02 to 5.45% while KOH-extractable alumimum varied between 0.03 and 3.24%. The relationship between the two forms of aluminum is expressed by the equation Y = 0.06 + 0.4404X where Y = KOH-Al (%) and X = acid oxalate Al (%), with and r = 0.982

Recientemente, Blakemore (2) describió un método sencillo para determinar aluminio activo en suelos derivados de cenizas volcánicas. La relevancia de esta fracción en la taxonomía de estos suelos estriba en su contribución a la retención de fósforo (1), a tal grado que ha sido considerado como criterio de clasificación por el Icomand (3).

La presente comunicación tiene como objetivo el proporcionar mayor información sobre la correlación entre dos métodos empleados para determinar aluminio activo en suelos de Costa Rica y Guatemala.

Materiales y métodos

En este trabajo se analizan 51 muestras de horizontes A, B y C de Andepts de Costa Rica y Guatemala. Las muestras, molidas en mortero hasta menos de 2 mm, representan Dystrandepts, Vitrandepts, Placandepts y Andaquepts y sus propiedades principales se encuentran en el Cuadro 1.

Cuadro 1. Variación de las características químicas de los suelos de este estudio.

Variable	Promedio ± Un error stándar	Ambito
pH (H_2O)	5.8 ± 3.8	4.8 – 6.9
pH (K Cl)	4.7 ± 3.6	3.7 – 5.3
Acidez Camb., meq/100 g	0.3 ± 0.3	tr – 2.0
K, meq/100 g	1.0 ± 1.1	tr – 7.3
Na, meq/100 g	0.2 ± 0.1	tr – 0.6
Ca, meq/100 g	3.0 ± 2.5	0.1 – 9.4
Mg, meq/100 g	1.1 ± 0.9	0.1 – 4.5
Al activo, %	2.2 ± 1.8	tr – 5.4
Fe activo, %	0.8 ± 0.6	tr – 2.8
Si activo, %	0.7 ± 0.6	tr – 2.2
pH Na F 2 min	9.4 ± 1.3	7.3 – 11.9
pH Na F 60 min	10.1 ± 1.1	7.7 – 12.1
Area Superficial, m^2/g	35.0 ± 53.0	1.0 – 292.0
Limo + Arcilla, %	55.8 ± 25.1	6.4 – 98.1
Materia Orgánica, %	9.2 ± 7.1	tr – 25.3
P Disponible, ppm	10.6 ± 10.9	tr – 48.9
P-Retenido	78.0 ± 17.0	25.1 – 99.6