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Soni Joshi
PG student, Department of Horticulture, College of Agriculture, G.B. Pant University, of Agriculture and Technology, Pantnagar, Uttarakhand, India

Ratna Rai
Professor, Horticulture, College of Agriculture, G.B. Pant University, of Agriculture and Technology, Pantnagar, Uttarakhand, India

AK Pant
J.R.O. Department of Soil Science, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Ranjan Srivastava
Professor, Horticulture, College of Agriculture, G.B. Pant University, of Agriculture and Technology, Pantnagar, Uttarakhand, India

JC Chandola
Ph.D. student, Department of Horticulture, College of Agriculture, G.B. Pant University, of Agriculture and Technology, Pantnagar, Uttarakhand, India

Correspondence
Ratna Rai
Department of Horticulture, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar, Uttarakhand, India

Variation in foliar nutrient status in relation to leaf age, position and sample size in sapota

Soni Joshi, Ratna Rai, AK Pant, Ranjan Srivastava and JC Chandola

Abstract
There has not been much research on variation in foliar concentration of nutrients in sapota as affected by leaf age, position and sample size of leaves. Standardized leaf sampling technique forms the basis for fertilizer recommendation in fruit crops. Therefore, in the present study, the effect of leaf age, position of leaves on the shoot and leaf sample size on the nutrient status of sapota cv. Cricket ball was studied. The leaf age and position of leaves on the shoot significantly influenced the leaf mineral composition. It was found that N, P, K, Zn, Cu contents decreased, whereas Ca, Mg, Fe and Mn contents increased with increasing leaf age. The N, P, K and Cu contents decreased from apical to basal leaves while Ca, Mg, Fe and Mn contents were more in the basal and middle leaves. The effect of position of leaves on Zn content remained non significant. The different sample sizes taken in the experiment did not exert significant effect on the mineral content of leaves except for Fe and Mn which increased with the sample size, however, the difference between 40 and 60 pairs of leaves was non significant. The interaction effect of position of leaf with sample size was significant for Fe, Mn and Cu while that between leaf age and position of leaves affected Cu content only. The results provide important information that the concentration of different nutrients changed with age, position on the shoot and sample size of sapota leaves. The data may be valuable for proper assessment of nutritional status of sapota plants and in the formulation of fertilizer doses eventually.

Keywords: Sapota, leaf analysis, mineral composition, foliar concentration

Introduction
Sapota (*Achras sapota* L.) an important fruit of the tropics is valued for its delicious and nutritive fruits. It is known by several names as sapota, chiku, ciku, dilly, nasberry, sapodilla plum, chico zapote, zapote, chico, néspero and sapota plum in different regions of the world (Yahia, 2014) [34]. India is the largest producer of Sapota in the world (Tsomu *et al.*, 2015) [32]. Besides India, it is one of the major fruit crop of Mexico, Guatemala and Venezuela. Sapota is a very hardy, highly productive and generally free from major pests, diseases and physiological disorders and so has emerged as an important fruit crop. Sapota fruits are priced for pleasant aroma and sweet taste. Sapota is a rich source of tannins, antioxidants, polyphenols alongwith anti- parasitic, anti- bacterial, anti- viral and anti- cancerous properties. Vitamin A present in sapota helps to ensure proper vision and vitamin C helps to strengthen the immune system. The unripe fruits and bark yield milky white latex which solidifies on exposure to air and is widely used in the preparation of Chickles. The cultivar, 'Cricket Ball' bears attractive large-round fruits with sweet, crisp and gritty pulp and pleasant flavour. Although sapota is essentially a tropical fruit crop but it is gaining popularity in the subtropical *tarai* region of Uttarakhand state due to production of quality fruits.
Optimal mineral nutrition of fruit trees play an important role in enhancing yield, growth and productivity of plants. Leaf nutrient analysis is the most effective guide for determining the nutritional status of a plant and forms the basis in determining future fertilization programmes (Singh *et al*., 2016, Sun *et al.*, 2015) [30, 31]. Leaf analysis acts as a very useful tool in the assessment of plant nutrient status provided the analytical data has been adequately interpreted (Bhargava and Chadha, 1993) [3]. Leaf analysis of plant materials determines level of sufficiency or deficiency of a particular nutrient for formulation of appropriate fertilizer recommendation and also for monitoring the effectiveness of current fertilizer practices (Jones *et al*., 1991, Kafkafi and Ganmore, 1997) [15, 16]. However, the effectiveness of leaf analysis may depend upon leaf age, its position on the shoot and size of the sample (Perica, 2001, Kumar and Singh, 2005) [23, 18].

Sapota tree produces several vegetative and floral flushes during the year and consequently the fruits require substantial amount of nutrients for maximizing yield and quality. It is, therefore, essential to see that the sapota trees are timely supplied with suitable and adequate nutrients for ensuring higher yields and sustained productivity. The past studies have shown that the nitrogen and potassium contents were lowest in the younger leaves in sapota while the phosphorous content remained stable throughout the year. The levels of various nutrients increased with age of leaves, however, wide fluctuation and sharp declines were noted in between during different periods of the year as the age of leaves increased (Chavan and Patil, 1980) [5]. In earlier investigations conducted on sapota, it was reported that leaves from the 10th position may be utilized for diagnosis of leaf nutrients status (Annapurna *et al*., (1988) [1]. However, no information is available on interactions among different leaf sampling factors like leaf age, its position on shoot and sample size in standardizing leaf analysis technique in sapota. Besides, comprehensive leaf nutrient diagnostic norms are not available for sapota in *tarai* subtropical region of Uttarakhand. The objective of the present investigation was to standardize the leaf analysis technique in sapota as affected by leaf age, its position and sample size.

Materials and methods

The experiment was conducted on twelve year old sapota trees of cv. Cricket Ball at Horticultural Research Station, Patharchatta, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar. Pantnagar is situated at 298°N latitude, 79.38°E longitude and at an altitude of 243.8 meters above the mean sea level in foothills of the Himalayas. The maximum temperature (39.2 ºC) was recorded during the month of May, whereas minimum temperature (5.9 ºC) was observed during the month of January. The sapota plants grafted on *khirni* rootstocks were spaced at 6m x 6m in square system of planting. Standard orchard management was practiced in all the trees taken under experiment. The trial was conducted in three factorial Randomized Block Design with 54 treatments, 4 replications and 100 leaves per replication. The three factors studied were leaf age, position of leaves on the shoot and sample size. The newly emerged shoots were tagged in April'2013. The samples were collected at bi monthly interval starting from June'2013 to April, 2014. from three positions i.e. apex, middle and base. The three sample size consisted of 20 pairs, 40 pairs and 60 pairs of leaves. Leaf samples were collected from all directions of plants and kept in brown paper bags. The leaves were brought to the laboratory and thoroughly washed with distilled water to remove the surface residues. The samples were then dried in hot air oven at 60 ± 2 ºC for 48 hours until stable weight was attained. Subsequently, the samples were powdered for further analysis. The leaf samples were analysed for N, P, K, Ca, Mg, Fe, Mn, Zn and Cu contents. Nitrogen was determined by Kjeldahl's method using Kelplus semi – auto analyzer nitrogen estimation system (M/s Pelican Equipment, Chennai). For the estimation of other elements, a diacid mixture comprising of concentrated nitric acid and perchloric acid (4:1 v/v) was prepared and 0.5 g sample was wet digested. Phosphorus was analysed by vandate - molybdate method suggested by Jackson (1967) [14]. Leaf K, Ca Mg, Fe, Zn, Cu, and Mn contents were determined by atomic absorption spectrophotometer (HITACHI 207). The expression of all the nutrients was made on dry weight basis.

All data were subjected to analysis of variance (ANOVA) and significant differences were determined at 5% level of significance (Gomez and Gomez, 1983) [10].

Results and discussion

Effect of leaf age

1. Nitrogen: The nitrogen content (Table 1) initially increased from June to August (2 and 4 month old leaves, respectively) and then gradually declined from October to April with increase in age (6 to 12 month older leaves). The highest mean nitrogen content was registered in four month old leaves (1.719%) while the minimum nitrogen content (0.848%) was found in the 12 month old leaves. The initial increase in nitrogen content might be due to faster expansion leaves and dry matter accumulation (Kumar and Singh, 2005) [18]. Thereafter, the decrease in nitrogen content may be attributed to consumption of nitrogen by the developing fruits for carrying out various physiological processes. Baloda *et al.*, (2004) [2] also agreed that the nitrogen content decreases with advancement of age due to its utilization for vegetative growth and fruit development. Nitrogen being an integral component of proteins, amino acids and membranes is required in higher concentration especially in those plant tissues where living cells are present in greater proportions (Clarkson and Hanson, 1980) [7]. Active metabolism in the developing tissues of younger leaves might have led to higher absorption of nutrients from soil and eventually its higher concentration.

2. Phosphorous: The phosphorous content (Table 1) remained statistically *at par* throughout the sampling period. However, it was higher (0.224%) in the younger 2 month old leaves (June sampling) and showed steady decline as the leaves matured and was minimum (0.168%) in the 12 month older leaves (April sampling). The decrease in phosphorus content with advancement in leaf age may also be correlated with simultaneous development of fruits leading to more remobilization of this element for fruits. Although phosphorus content decreased with leaf age, but the difference between various ages was non significant. Elements like P do not accumulate in the mature leaves, but are transferred to the growing points (Guha and Mitchell, 1966) [11].

3. Potassium: The highest potassium content (1.595%) was noted in the 2 month old leaves (Table 1) and the concentration steadily decreased as the age of leaves increased. Lowest potassium content (0.825%) was observed in the 12 month older leaves. Decrease in potassium content of leaves with aging has also been reported by Chavan and Patil (1980) [5] in sapota. Elements like phosphorus and potassium have greater mobility in the phloem (Marschner, 2012) [21] and thus have a tendency to decrease with the age of the leaves. The decline in the potassium content with the advancing leaf age may be due to remobilization of this element in the younger leaves and to the developing fruits as well.

4. Calcium: Ca plays vital role in the physiological processes of a plant. There was significant influence of leaf age on the calcium content of sapota leaves (Table 1). The calcium content increased progressively as the leaves aged from 2 month to 12 month. There was slight reduction in the calcium content in the 8 month old leaves. The maximum calcium level (1.698%) was observed in the leaves which were 12 month old while the lowest calcium content (1.131%) was

observed in the youngest (two month old) leaves. Chavan and Patil (1980) [5] also found that the youngest leaves had lowest calcium content while the oldest leaves had highest calcium content in sapota. Calcium concentration in the plant is largely governed by new flushes and flowering pattern in the case of sapota (Savita and Anjaneyulu, 2008) [27]. The increase in the calcium content with the advancement of age might be due to the accumulation of calcium in the leaves and its immobile nature (Baloda *et al.,* 2004) [2].

5. Magnesium: The leaf magnesium content increased as the leaves matured (Table 1). The maximum magnesium content (0.825%) was recorded in the last sampling month (12 month older leaves) while the minimum Mg content (0.428%) was observed in the younger 2 months older leaves. Chavan and Patil (1980) [5] have also reported lowest magnesium content in young and tender leaves of Sapota. The increase in magnesium content with the advancement of leaf age might be due to the accumulation of magnesium in storage organs and its immobile nature in the plant system (Baloda *et al.*, 2004) [2].

6. Iron: The highest iron content (259.991 ppm) was recorded in twelve months old leaves, while the lowest (93.858 ppm) was found in the four month old leaves. The iron content initially decreased from first sampling to second sampling, however, the decline was found to be non-significant. Thereafter, it consistently increased up to the last sampling (Table 1). Significant increase in iron content was observed between fourth to twelfth month. This increasing trend might be due to low requirement of Fe by sinks and thereby, its retention in the donor tissue (Thakur and Rehalia, 2013) [33]. In Jackfruit also, variation in iron content with age of the leaves was observed (Sun *et al.* 2015) [31].

7. Manganese: The leaf age significantly influenced the manganese content of leaves (Table 1). Manganese content increased with the age of leaves. In the last sampling, highest Mn content (81.752 ppm) was recorded while the lowest was found in the two months older leaves (51.148 ppm). Kumar and Singh (2005) [18] have also reported that in general, manganese content increases as the leaf age advances. This might be due to its high rate of uptake from the soil and immobility within the plant system.

8. Zinc: As the leaves matured, the zinc concentration decreased (Table 1). The maximum zinc content (19.266 ppm) was observed in two months old youngest leaves, whereas minimum zinc content (11.171 ppm) was recorded in 12 months older leaves. The decline in zinc concentration between all the months was found to be significant except in fourth and sixth month. The insufficient movement of zinc from younger leaves to growing parts might have led to decline in the zinc content in the older leaves (Reddy and Tiwari, 1985) [25].

9. Copper: The copper content decreased as the age of leaves increased (Table 1). The maximum leaf copper content (14.368 ppm) was recorded in youngest two months older leaves while minimum copper content (8.131 ppm) was observed in the oldest leaves. Ageing of leaves resulted in reduction in the level of copper. Earlier workers (Diaz and Romo, 1988; Clark *et al.*, 1989) [9, 6] have also reported progressive decline in the copper level of leaves as the season advances. The reduction in the copper concentration might have been due to its remobilization towards the new developing fruits taking place simultaneously.

Effect of position of leaves

1. Nitrogen: The apical leaves showed significantly higher nitrogen content (1.350%) as compared to middle and basal leaves (Table 2). There was non-significant difference in the nitrogen content of the middle (1.116%) and basal (1.115%) leaves. The reason behind this might be attributed to the fact that nitrogen being a mobile element has the tendency to accumulate in the younger leaves situated at apical position (Singh and Rajput, 1978) [29].

2. Phosphorous: The maximum phosphorous content was again observed in apical leaves (0.383%), followed by middle (0.098%) and basal leaves (0.087%).The difference between phosphorus content of middle and basal leaves was, however, non significant. Guha and Mitchell (1966) [11] observed that mobile elements like phosphorous do not accumulate in mature leaves but are transferred to the growing points and developing fruits.

3. Potassium: The maximum potassium content (1.359%) was observed in the apical leaves (Table 2) followed by basal (1.111%) and middle leaves (1.083%). Potassium concentration in the apical leaves was significantly higher than its concentration in the middle and basal leaves which were statistically *at par*. Potassium is mobile in nature and gets accumulated in terminal leaves as compared to the middle and basal leaves (Guleryuz *et al.,* 1995) [12]. The tendency of potassium to accumulate in the terminal leaves due to its mobile nature has also been confirmed by Chandel and Rana (2004) [4] in kiwi fruit.

4. Calcium: The highest calcium content (1.542%) was observed in the basal leaves (Table 2) followed by middle (1.367%) and apical (1.342%) leaves. The calcium content in the apical and middle leaves was *at par*. Calcium is immobile in nature and tends to accumulate in the older leaves (Chavan and Patil,1980; Baloda *et al.*, 2004) [5, 2].

5. Magnesium: The maximum magnesium content (0.791%) was observed in the basal leaves (Table 2) followed by middle (0.556%) and apical leaves (0.546%). The difference between apical and middle leaves, however, was found to be non significant. Similar results have been obtained by Kumar and Pandey (1979) [19] in guava cv. Lucknow-49. The findings are also in conformity with the results of Hewitt (1955) [13] in Banana and Pathak and Pandey (1976) [22] in mango.

6. Iron: The basal leaves recorded maximum (173.646 ppm) iron content which was *at par* with that of middle leaves (Table 2). Minimum iron content (152.677 ppm), on the other hand, was observed in the apical leaves. This pattern might be due to the partial or incomplete mobility of this element (Sanchez-Alonos and Lachica 1987) [26]. Cummings (1977) [8] also observed highest iron content in the basal and middle leaves in grapes.

7. Manganese: The maximum manganese content (67.888 ppm) was observed in middle leaves (Table 2) and was *at par* with the Mn concentration in basal leaves (67.555 ppm). The lowest level of Mn was found in apical leaves (62.799 ppm). Thakur and Rehalia, (2013) [33] observed that middle leaves showed less fluctuation for manganese content in comparison

to apical and basal leaves with significantly higher value. Limited phloem mobility of manganese might be an important reason behind this trend (Labanauskas *et al.,* 1959) [20].

8. Zinc: The effect of position of leaves on zinc content of leaves was found to be non significant.

9. Copper: The maximum leaf copper content (12.074 ppm) was recorded in the leaves sampled from apical position followed by middle (11.004 ppm) and basal leaves (10.578 ppm). The difference between apical, middle and basal leaves was significant for copper content (Table 2). Sharma and Rehman (2012) [28] suggested partial mobility of copper in the plant system. Decline in copper content with advancement in season has also been reported by some workers (Diaz and Romo, 1988; Clark *et al.,* 1989) [9, 6].

Effect of Sample Size

Sample size had non-significant effect on N, P, K, Ca, Mg, Zn and Cu contents. It only affected iron and manganese contents (Table 3). The maximum amount of iron (172.100 ppm) was observed in the sample size consisting of 60 pair of leaves and was *at par* with the value for 40 leaf pair. The minimum iron content (156.946 ppm) was recorded in the sample size comprising of 20 pair of leaves. It is clear that as the sample size increased the leaf iron content also increased. Significant variation in iron content with different sample sizes has also been observed in apple cv. Red Delicious by Rawat and Singh (1991) who observed that sample size of 30-50 leaves was more appropriate for sampling as compared to 10 and 20 leaves. The maximum leaf manganese content was also observed with 60 pairs of leaves (67.933 ppm) and was *at par* with 40 pairs of leaves (66.844 ppm). The sample size comprising of 20 pairs leaves recorded significantly lower Mn content (63.465 ppm). Rawat and Singh (1991) [24] also observed that the variation in sample sizes significantly affected the manganese content in apple cv. Red Delicious.

Effect of Interaction

The interaction effect of leaf age and position of leaves was found to be significant for copper content only (Table 4). The maximum copper content (15.892 ppm) was found in two month old leaves collected from apical position whereas the minimum copper content (7.774 ppm) was observed in twelve month old leaves taken from middle position. It was found in general that the difference between middle and basal position for all leaf ages was non significant for copper content suggesting that there was stability in the copper content between these two positions.

The interaction effect of position of leaves and sample size was found to be significant for Fe, Mn and Cu content (Table 5). The maximum iron content (174.282 ppm) was recorded in 60 pair of leaves taken from basal position while minimum (127.463 ppm) was observed in 20 pair of leaves collected from apex position. The Mn content was found highest (68.607 ppm) in the 60 pair of leaves collected from basal portion of the shoot while lowest (54.505 ppm) was found in 20 pairs of leaves taken from apex position. Similarly, copper content was highest (12.367 ppm) in 60 pair of leaves collected from apical position.

The experimental findings suggest that leaf age and position of leaves significantly influenced levels of nutrients analyzed, whereas the sample size was significant only for iron and manganese. The leaf N, P, K, Zn and Cu contents decreased, in general with advancement in leaf age, however, the levels of Ca, Mg, Fe and Mn increased with maturity of leaves. The results indicated that different nutrients are undergoing flux in different seasons. Relative stability of K, Mg, Zn and Cu was recorded in the four to six months older leaves. The N, Ca and Mn contents showed stabilization in the six to eight months older leaves. In the P and Fe contents, relative stability was observed in the eight to ten months older leaves. Leaves collected from middle to basal portion of the shoots showed better stability of nutrients with sample size of 40-60 leaf pair giving relatively consistent results. Sharp reductions in the nutrient concentrations might be due to their depletion by the developing fruits. However, greater fluctuations in the leaf nutrient status of sapota during the rapid phase of vegetative and fruit growth are difficult to explain. Therefore, further research is required to correlate the leaf nutrient variations in relation to various physiological processes occurring during phenological cycle of sapota plants.

Table 1: Effect of Leaf age on mineral composition of sapota leaves

Leaf Age	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
Two month (M_1)	1.293	0.224	1.595	1.131	0.428	103.088	51.148	19.266	14.369
Four month (M_2)	1.719	0.198	1.420	1.283	0.513	93.858	57.730	17.816	13.001
Six month (M_3)	1.173	0.196	1.285	1.458	0.531	148.989	63.857	16.961	12.104
Eight month (M_4)	1.117	0.179	1.078	1.400	0.713	180.302	68.639	15.113	10.676
Ten month (M_5)	1.012	0.178	0.904	1.531	0.776	208.616	74.359	13.285	9.032
Twelve month (M_6)	0.848	0.168	0.725	1.698	0.825	259.991	81.752	11.171	8.131
C.D. at 5%	0.046	0.016	0.115	0.048	0.028	12.767	4.598	0.914	0.560

Table 2: Effect of position of leaves on mineral composition of sapota leaves

Position of leaves	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
Apical portion (P_1)	1.350	0.383	1.359	1.342	0.546	152.677	62.799	15.635	12.074
Middle portion (P_2)	1.116	0.098	1.083	1.367	0.556	171.099	67.888	15.430	11.004
Basal portion (P_3)	1.115	0.087	1.111	1.542	0.791	173.646	67.555	15.741	10.578
C.D. at 5%	0.033	0.012	0.029	0.036	0.015	9.027	3.251	NS	0.396

Table 3: Effect of sample size on mineral composition of sapota leaves

Sample Size	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
20 pairs (N_1)	1.270	0.494	1.262	1.455	0.706	156.946	63.465	15.337	11.080
60 pairs (N_3)	1.119	0.314	1.108	1.366	0.557	172.100	67.933	15.770	11.299
C.D. at 5%	NS	NS	NS	NS	NS	9.027	3.251	NS	NS

Table 4: Effect of interaction of leaf age and position of leaves on copper content of sapota leaves

Leaf age x Position of leaves	Two month (M_1)	Four month (M2)	Six month (M3)	Eight month (M4)	Ten month (M5)	Twelve month (M6)
Apical portion (P_1)	15.892	14.233	13.284	11.186	9.410	8.441
Middle portion (P_2)	13.687	11.963	11.212	10.171	8.663	7.774
Basal portion (P_3)	13.527	12.808	11.816	10.672	9.024	8.180
C.D. at 5%	0.970					

Table 5: Effect of interaction of position of leaf and sample size on mineral composition of sapota leaves

Position of leaves x Sample Size	Fe (ppm)	Mn (ppm)	Cu (ppm)
20 pairs (N_1) from Apical position (P_1)	127.463	54.505	10.138
20 pairs (N_1) from middle position (P_2)	170.890	68.284	11.555
20 pairs (N_1) from basal position (P_3)	172.484	67.608	11.546
40 pairs (N_2) from Apical position (P_1)	159.773	65.285	10.767
40 pairs (N_2) from middle position (P_2)	171.183	67.716	12.301
40 pairs (N_2) from basal position (P_3)	174.172	67.531	10.767
60 pairs (N_3) from Apical position (P_1)	170.795	67.528	12.367
60 pairs (N_3) from middle position (P_2)	171.224	67.665	10.829
60 pairs (N_3) from basal position (P_3)	174.282	68.607	10.701
C.D. at 5%	15.636	5.632	0.686

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