

## EFFECT OF N, K, CI ON PHOTOSYNTHESIS AND WATER RELATIONS OF OPEN POLLINATED TALL (*typica*) COCONUT SEEDLINGS

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### ABSTRACT

CO<sub>2</sub> assimilation and plant water status were measured in sand-culture-grown amputated coconut seedlings treated with three levels of N, K, and C1 and subjected to different soil water deficit conditions. Application of increased levels of N and K enhanced the assimilation of CO<sub>2</sub> while individual and combined effects of N, K, and C1 improved the water economy of coconut seedlings. Under the soil water deficit conditions, adequate supply of N helped to maintain high leaf water potential by the accumulation of solutes like proline and sugars. Whereas, adequate supply of K and C1 themselves acted as osmotica by increased accumulation of these nutrients in leaf tissues, although other solute concentrations such as those of sugars and proline were reduced. Our data show that maintenance of sufficient levels of N and K contribute to the growth of the coconut seedling through improved gaseous exchange, CO<sub>2</sub> assimilation and better partitioning of assimilated carbon into shoot and roots. potassium and C1 are further important for maintenance of water status of coconut seedlings by improved stomatal regulation, water uptake and osmotic adjustment of tissues under water deficit conditions.

### INTRODUCTION

Productivity of perennial tree crops mainly depends on climatic factors, availability of soil nutrients and fertilizer usage in addition to their genotypic characters. Nitrogen is an important nutrient for coconut, specially during the seedling stage. It is essential because if combines with organic molecules to form biologically active compounds like amino acids, proteins, nucleic acids, phyto hormones etc. Nitrogen deficiency in coconut palms manifests itself by yellowing of leaves, reduced vegetative growth, diminished female flower production and yield.

It has been reported that potassium and magnesium are the two most limiting nutrients affecting the productivity of coconut palms in Sri Lanka with about 80% of the coconut plantations showing low leaf nutrient levels (Jayasekara, 1989). Potassium ion is found to be the only ion which has a major effect on the diffusion of CO<sub>2</sub> to the chloroplast in mesophyll cells. Peaslee and Moss (1968) and Terry and Ulrich (1973) reported that decline in rate of photosynthesis of maize and sugar beet leaves as potassium become deficient. Potassium further influences protein synthesis (Hsiao et al., 1970), water uptake by roots (Mangel and Kirkby, 1980), cell turgor maintenance (Leigh and Wyne Jones, 1984), water transport and enzymatic reactions. Potassium in coconut is mainly stored in the husks, nut water, leaves and petioles. The average annual removals of potassium from those crop components were found to be approximately 0.75 to 0.9 kg palm<sup>-1</sup> y<sup>-1</sup> (Nathaniel, 1969).

Until recently, the importance of chlorine as a plant nutrient in coconut was not known. However, recent studies have shown that chloride ions are essential for certain genera of plants including palmae (Von Uexkull, 1984). Potassium and chloride ions in plant cells do not complex with organic molecules but function as free ions. Potassium in plant cell exists compartmentalized as cytoplasmic and vacuolar  $K^+$ . These subcellular compartments have different  $K^+$  concentrations (Leigh and Wyne Jones, 1986). Cytoplasmic potassium is essential for biochemical processes such as photosynthesis, glycolysis, starch and protein synthesis, whereas vacuolar potassium mainly act as an osmoticum. Stomatal regulation for gaseous exchange and transpiration depend on the turgor and solute potential of guard cells.

In this paper importance of N, K, and Cl for two main physiological processes namely (a)  $CO_2$  assimilation and (b) water relations of the coconut seedlings are discussed. The processes of transpiration (E) and of  $CO_2$  assimilation (A) both are crucial in plant productivity and depend on  $CO_2$  and water vapour diffusion through stomata. Stomatal regulation of gaseous exchange takes place by opening and closing of stomatal aperture, which is stimulated by turgor mediated process within guard cells and potassium play a key role as the osmotic agent for turgor changes. Variation in stomatal aperture in relation to the rate of transpiration or  $CO_2$  assimilation are quantified at the leaf or whole plant level as changes in either stomatal diffusive resistance (g) or conductance (Reciprocal of g). Measure of these parameters in the field or glass house level could be used as an indicator of either water status, photosynthesis or transpiration rates of plants. Similarly water status of plants could be measured as water content (WC), relative water content (RWC), and water potential ( $\psi$ ) of tissues. Eventhough, WC and RWC quantify the amount of water present in a plant tissues they do not imply the driving force for water within tissues. In simple terms water potential ( $\psi$ ) is the driving force for water movement in liquid phase within the soil-plant system. Presently pressure chamber and psychrometers are widely used to measure plant and soil water potentials. In this study pressure chamber has been used to measure leaf water potential of coconut leaves.

## MATERIAL AND METHODS

### Material :

Open pollinated *typica* coconut seednuts were obtained from the Bandirippuwa estate, Lunuwila and seedlings were grown from dehusked seednuts in nursery beds. Plastic containers (30 cm in diameter and 34 cm in height) were filled with washed sand. Seedlings were amputated from the seednuts, eight weeks after sprouting. Any damaged roots were cut and dipped in a 5% benlate solution (fungicide) for 10 minutes. Cut ends were covered with a panel dressing Tb - 192 (CIC Ltd) and seedlings were transferred into the containers of washed sand.

### Experimental :

Experiment was a  $3^4$  factorial confounded block design with nine blocks and each block with nine replicates. Three levels each of N, K, and Cl as 0 mM, 5 mM, and 10 mM were used as the nutrient treatments. Half strength Hoagland solution was used to supply other macro and micro nutrients. In 0 mM N, K, and Cl treatments ionic strength of the Hoagland solution was balanced by substitution of Na and  $SO_4$  ions. Nutrients were applied three times a week and seedlings were watered to the field capacity with deionized water for ten months. Two drying cycles were imposed to simulate drought conditions. One set of seedlings was dried up to six weeks and the other set was dried for eight weeks by withholding application of water. A third set was maintained at the

field capacity by regular watering throughout the study. At the end of each drying cycle, seedlings were rewatered up to the field capacity. Whenever necessary few leaflets were harvested from last fully expanded leaf for  $\psi_1$ , chlorophyll, and amino acid and sugar assay.

## METHODS

### Chlorophyll concentration :

Chlorophyll in fresh leaf samples was extracted homogenizing in 20 ml of 80% acetone (v/v) in an ultra turrex. After centrifugation (1500 rpm, 5 min) at room temperature, absorbance of the supernatant fraction was measured at 645 and 668 nm in a UV/VIS spectrophotometer (Shimadzu UV 160- A, Japan).

### CO<sub>2</sub> assimilation :

The rate of CO<sub>2</sub> assimilation under planthouse conditions was measured using a portable ADC photosynthesis meter (Analytical Development Co. Ltd., Hertfordshire, U.K.). The instrument was operated in the differential mode at the normal environmental conditions.

### Leaf water potential and stomatal diffusive resistance :

Water potential of leaves was measured using a commercial type pressure chamber (Soil Moisture Corporation, California, U.S.A.). Stomatal diffusive resistance and rate of transpiration of leaves were measured using a Li-1600 steady state porometer (LI-COR INC., Nebraska, USA)

### Sugars and proline :

Dried ground leaf samples (0.5 g) were homogenized in an ultra turrex with 20 ml of 80% ethanol (v/v) and with the use of chloroform-water, chlorophyll was separated from the aqueous fraction. Aqueous fractions were then centrifuged (1500 rpm, 5 min) and supernatant fractions were then centrifuged (1500 rpm, 5 min) and supernatant fractions were concentrated to a constant volume of 5 ml. Total sugars in the concentrated fractions were determined by the phenol-sulphuric assay method (Dubois et al., 1956).

Reducing sugars in the aqueous fractions were determined by the 3-5 dinitrosalicylic acid method as described by Sumner (1924).

Fresh leaf samples (5 g) were homogenized with 80% ethanol in an ultra turrex and the soluble fractions were extracted as described above and proline concentration was determined by the method of Troll and Lindsay (1955) without using permutit-T resin.

## RESULTS

### Effect of N,K, and Cl on Chlorophyll content:

The effects of different treatment levels of N,K, and Cl on leaf chlorophyll concentration as well as the chlorophyll a/b ratio are given in the Table 1. Analysis of leaf nutrients revealed that N,K, and Cl exceeded the critical levels when the treatment nutrient levels were increased from 5 mM to 10 mM. High N levels increased the chlorophyll a & b concentrations. Nevertheless, chlorophyll a/b ratio was decreased significantly. Potassium has no effect on leaf chlorophyll

concentration. Increased levels of chlorine reduced the chlorophyll concentration possibly by reducing synthesis.

### **The effect on CO<sub>2</sub> assimilation and assimilate partitioning :**

CO<sub>2</sub> assimilation, stomatal conductance and inter cellular CO<sub>2</sub> concentration of the second expanded leaf of the individual seedlings were determined at field capacity when the seedlings were 8,9, and 10 months old. Fig. 1 shows the relationship between rate of photosynthesis and transpiration of coconut seedling at field capacity and with different levels of N,K, and Cl for averaged data for three consecutive measurements. Increased supply of N and K enhanced the rate of CO<sub>2</sub> assimilation, whereas chlorine showed a negative response by reducing assimilation of CO<sub>2</sub>. Increased supply of K and reduced supply of Cl increased the leaf water potential (less negative) of seedlings with concomitant increase in the rate of CO<sub>2</sub> assimilation. Increased supply of N increased the assimilation of CO<sub>2</sub> of leaves but reduced the leaf water potential of coconut seedlings.

### **The effect on water relations :**

Leaf water potential ( $\psi_2$ ), rate of transpiration (E), and stomatal diffusive resistance (g) of the same leaves used for CO<sub>2</sub> assimilation studies were measured concurrently. Fig. 2 shows the relationship between  $\psi_1$  and E with different levels of N,K, and Cl. Nitrogen and K have shown linear and contrasting effects on leaf water potential, whereas transpiration was increased with increase in the supply on N and K. Tables 2, 3, and 4 describe the effect of interaction between of K and Cl, N and Cl, and N and K on leaf water potential, transpiration, and stomatal diffusive resistance of seedlings. All three nutrient combinations displayed significant interactive effects either enhancing or decreasing their response to these parameters depending on the levels of nutrients.

### **The effect of N,K, and Cl on osmotic adjustment :**

Some plants have the ability to maintain tissue turgor at higher levels under soil water deficit conditions by accumulation of solutes within the tissues. Sugars, sugar acids, cations and anions like k, Na, Cl, malate, imino acids like proline and glycinebetaine are some such substances accumulated within tissues to maintain turgor balance. Table 5 shows the accumulated levels of sugars, proline, K, and Cl under water deficit conditions. It was very clear that in the presence of adequate supply of N, leaf sugar and proline concentrations were increased at soil water deficit conditions, whereas in the presence of adequate supply of K and Cl, proline concentration was decreased but concentrations of these ions themselves were increased in leaves. These results suggest that K and Cl help to maintain turgor balance under water deficit conditions.

## **DISCUSSION**

Nitrogen is an important nutrient for plant growth. As in many other plants N has increased chlorophyll content and photosynthetic activity of coconut seedlings. Nitrogen deficiency results in reduction of stomatal conductance (Balton and Brown, 1980) or mesophyll conductance (Wong, 1979) or both (Navin and Loomis, 1970; Brown and Wilson, 1983). Jayasekara (1986) reported that the reduction in leaf water potential, stomatal conductance, and photosynthetic activity under nitrogen deficiency and water deficit conditions in *Chamaedorea elegans* palms. Similarly nitrogen deficiency reduces the stomatal conductance and photosynthetic activity in coconut seedlings. Nitrogen metabolism is inhibited under reduced water potentials. Synthesis of

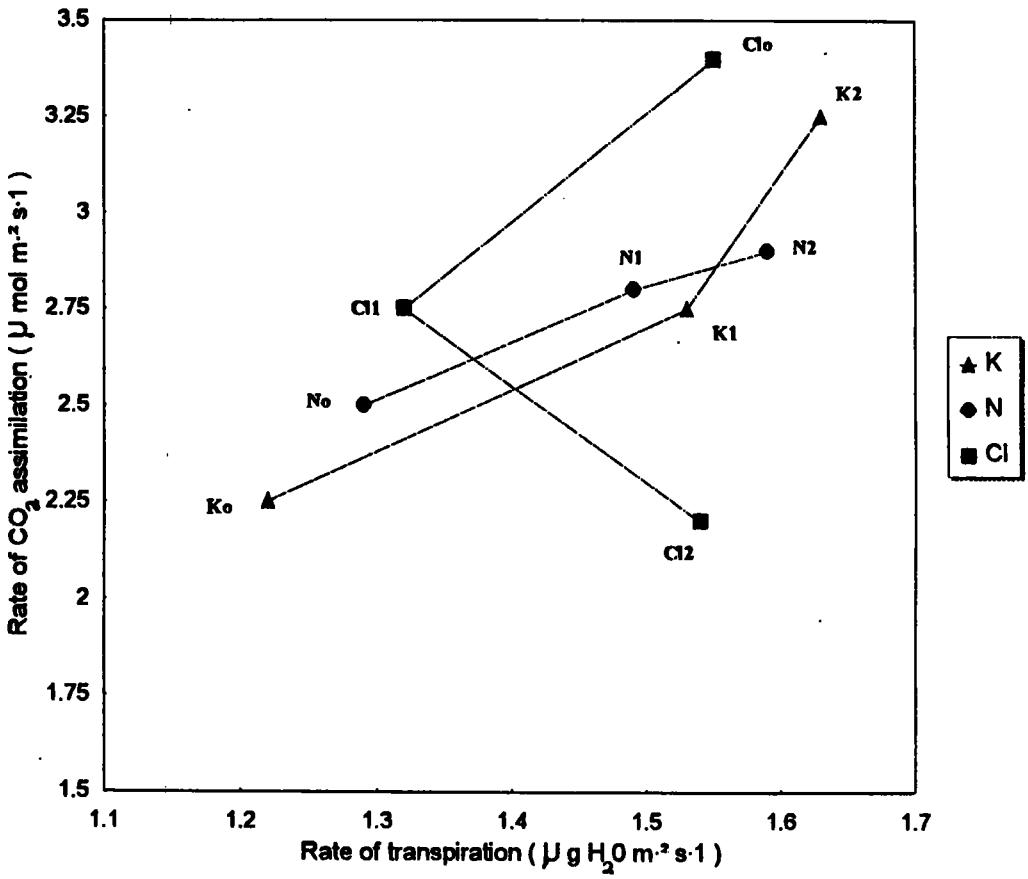


Figure 1 : *Relationship between rates of transpirations and rate of photosynthesis with different treatment levels of N (●—●), K (▲—▲) and Cl (■—■).*

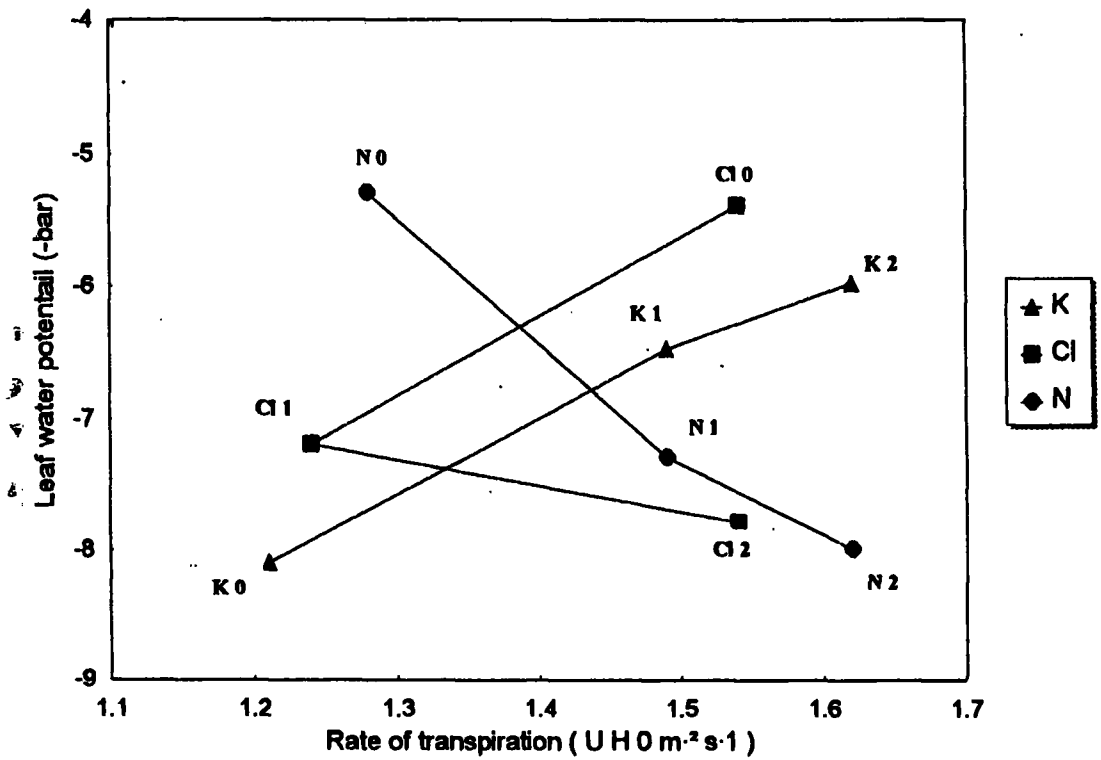


Figure 2 : Relationship between rate of transpiration and leaf water potential with different treatment levels of N (●—●), K (▲—▲), and Cl (■—■).

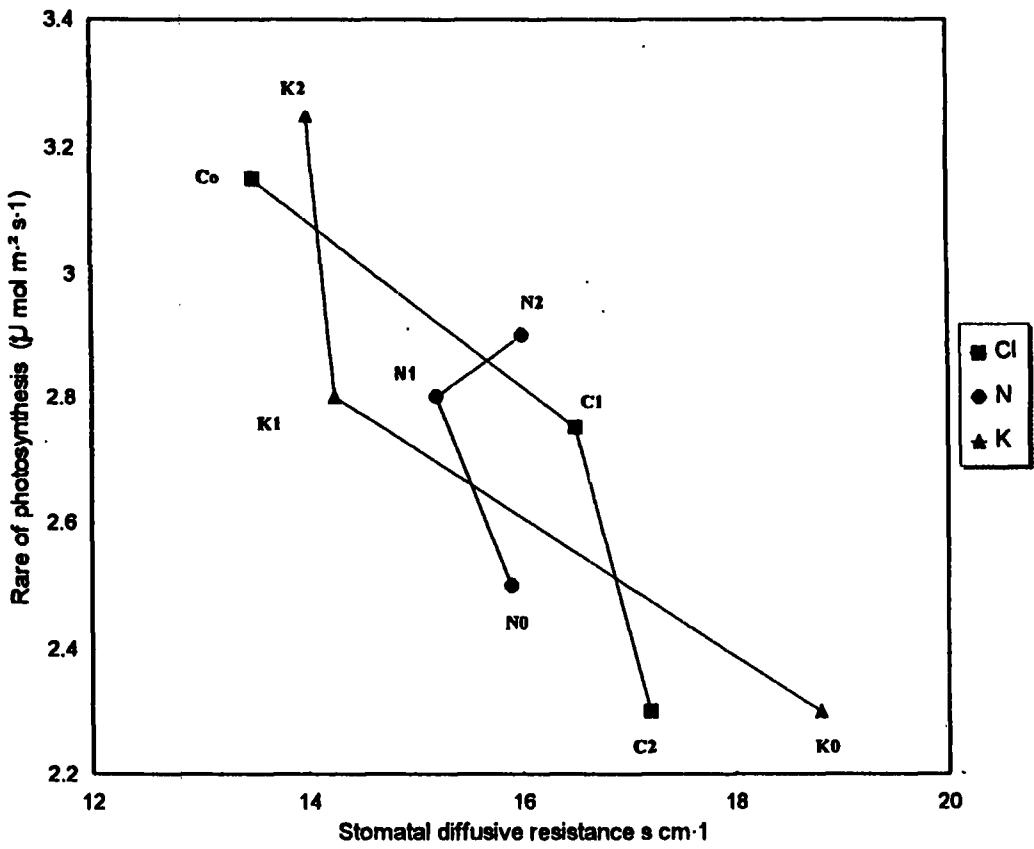


Figure 3 : Relationship between rate of photosynthesis and stomatal diffusive resistance under different levels of N (●—●), K (▲—▲), and Cl (■—■).

proteins inhibited and hydrolysis of existing proteins is enhanced, consequently increasing the concentration of free amino acids or other nitrogen compounds. This is specially so in the case of the imino acid proline. In this study it became apparent that under water deficit conditions, total leaf amino acids and proline concentrations were increased with increase in the nitrogen supply.

Gaseous exchange of water vapour and  $\text{CO}_2$  in leaves largely depend on the movement stomatal guard cells. During the opening and closure of stomata, K plays a pivotal role as an osmotic agent for turgor changes in the guard cells. Humble and Raschke (1971) provided convincing evidence for the requirement of  $\text{K}^+$  for stomatal movement by electrone probe analysis. Very recently by the same method, Serge Braconnier and Jean d'Auzac (1990) reported, that on stomatal opening, increase of  $\text{K}^+$  and  $\text{Cl}^-$  in guard cells of coconut and oil palm leaves and their concentration variation percentage of  $\text{K}^+$  and  $\text{Cl}^-$  were quite similar for both species of palms. In general stomata open less under K-deficiency, but this may occur only at an advanced stage of deficiency in many plants (Hsiao, 1975). As presented in Fig. 3, higher levels of K showed a marked increase in assimilation of  $\text{CO}_2$  probably through increased stomatal conductance for gaseous exchange and maintenance of high water potential within leaves. Further, requirement of K for the photophosphorylation process within chloroplasts (Trebst, 1980) and promotion of RuBp-carboxylase enzyme synthesis (Peoples and Koch, 1979) has been reported for other plants. However, such effects were not investigated as they were beyond the scope of this study. Uptake of  $\text{K}^+$  by root cells depresses the osmotic potential of the cells, thereby inducing uptake of water from soil. Water transport into the xylem vessels is also an osmotic process, function of  $\text{K}^+$  in which is very important for upward movement of water. Therefore,  $\text{K}^+$  is the important osmoticum which drives water flux from the surrounding cells into the xylem vessels (Baker and Weatherly, 1969). In this study it was observed that K and Cl accumulated in leaf tissues have possibly acted as osmotica to maintain the turgor balance under water deficit conditions. In the treatments where high concentration of potassium, high transpiration rates and  $\psi$ , were maintained. Thus from these observations it was clear that K in coconut seedlings has helped to regulate turgor potential within tissues, maintain water balance or water economy by influencing water uptake, upward movement and transpiration through stomata. At the same time  $\text{K}^+$  helps to maintain high photosynthetic activity in coconut seedlings. These beneficial effects of K in coconut seedlings are particularly important for crop production if they hold also for native palms.

Chlorine deficiency in coconut leaves manifests itself by drying of leaves at the base as a result of inadequate supply of water (Ollagnier, 1985). Several authors have reported that the beneficial effects of Cl on yield, drought tolerance induced by K and Cl, and improvement of assimilation of  $\text{CO}_2$  (Magat et al., 1975; Von Uexkull, 1984; Ollagnier, 1985). Increased yield and growth of coconut and oil palm were observed when leaf Cl concentration was brought up to the critical levels of 0.65% of dry weight. However, in this study we found that Cl concentration in the zero chlorine treatments was in the range of 0.40 - 0.90% depending on the N, K, and water treatments.

High concentrations of chlorine in the zero treatments probably resulted from the tissue Cl levels at the time of amputation of seedlings and also from Cl received from the atmosphere (wind borne from the coast). Therefore, the effect of low levels of Cl supply (below critical level) on physiological processes of coconut palm seedlings was not well reflected in this study. High tissue Cl concentrations (above critical level) resulted in undesirable effects on growth of coconut seedling by reducing chlorophyll content and assimilation of  $\text{CO}_2$ . However, interactive effects of K and Cl gave beneficial effects by improving osmotic adjustment by turgor balance and leaf water potential under water-limiting conditions.



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