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$$M_{x-1}^{\cdot} + M \xrightarrow{k_p} M_x^{\cdot}$$

$$M_x^{\cdot} + \text{complex} \xrightarrow{k_t} \text{polymer} + Mn^{2+} + Z$$

where Z may be L-TRN itself or an inactive product obtained from L-TRN (M = AAM). Using the steady-state assumption, the following expressions are derived

$$R_p = \frac{k_p \cdot k_d [\text{AAM}]^2}{k_t ([\text{AAM}] + (k_o/k_t)[Mn^{3+}])} . \quad (1)$$

The value of the term $(k_o/k_t)[Mn^{3+}]$ is negligibly small (0.0177) when compared to the value of [AAM]. Hence neglecting $(k_o/k_t)[Mn^{3+}]$ in (1), the following equation is obtained

$$R_p = (k_p \cdot k_d [\text{AAM}])/k_t . \quad (2)$$

$-R_m$ is given by the expression

$$-R_m = \frac{2Kk_d [Mn^{3+}]_{\text{total}} [\text{L-TRN}]}{1 + K[\text{L-TRN}]}, \quad (3)$$

where $[Mn^{3+}]_{\text{total}} = [Mn^{3+}] + K[Mn^{3+}][\text{L-TRN}]$.

The expressions (2) and (3) agree with our observations. Various kinetic and thermodynamic parameters have been evaluated and presented:

$\Delta E_a^{\neq}$ k cal.mol^{-1}	$\Delta S^{\neq}_{313^\circ K}$ e.u. mol^{-1}	$\Delta G^{\neq}$ k cal. mol^{-1}	k_p/k_t	k_o/k_t
3.32	−67.30	24.39	0.9966	6.731

19 September 1987

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A NEW ISOLATE OF *PLASMODIUM FALCIPARUM* AND ITS CHLOROQUINE SENSITIVITY

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MALARIA caused by the protozoan parasites of genus *Plasmodium* has been identified as a major public health problem in many developing countries including India[1]. Falciparum malaria due to *P. falciparum* is the most fatal form of the disease and also involves cerebral, renal and pulmonary complications. Although the overall incidence of malaria, after an upsurge in 1970's, has shown a decreasing trend in recent years (2 million cases in 1984), the incidence of *P. falciparum* has proved less responsive and constitutes a higher proportion of the total malaria cases (30% in 1984 as compared to 10% in 1977)[2].

The chloroquine-resistant strain of *P. falciparum* has emerged as the major setback to chemotherapy necessitating major international efforts. But the mechanism of antimalarial drug action as well as biochemical and genetic mechanisms of resistance are not known[3,4]. Studies on different geographical isolates of *P. falciparum* and their drug sensitivity are essential to understand the mechanism and spread of drug resistance as well as to evolve suitable chemotherapeutic measures. Studies on different isolates of *P. falciparum* outside India have shown the existence of genetic diversity as judged by isoenzyme and antigenic pattern as well as the sensitivity to anti-malarial drugs[5,6]. To initiate such studies on Indian isolates of *P. falciparum*, the adaptation of isolates from different regions of India, to *in vitro* culture, is a pre-requisite. Three Indian isolates of *P. falciparum* (FAN-5 from Rajasthan[7], MRC from Haryana[8] and FCK-2 from Karnataka[9]) have been adapted to *in vitro* culture and used for serological and seroepidemiological studies.

In the present study we have established an isolate (DCK-1) of *P. falciparum* (from Lucknow, UP) in *in*

Table 1. *Chloroquine sensitivity of Plasmodium falciparum isolates by micro in vitro test*

Isolates	Effective dose (p mol/μl of blood)			
	50.0	90.0	95.0	99.9
DCK 1	0.247 ± 0.011	0.336 ± 0.011	0.417 ± 0.021	0.494 ± 0.003
Z	0.529 ± 0.025	0.739 ± 0.010	0.808 ± 0.021	0.923 ± 0.024
KZ1	2.257 ± 0.159	6.249 ± 0.208	8.541 ± 0.208	10.208 ± 0.208
Ituxi 084	13.604 ± 0.219	21.527 ± 0.601	24.375 ± 0.551	26.597 ± 0.524

Data are mean ± SD of six replicates.

vitro culture and compared its chloroquine sensitivity vis-a-vis with other isolates of *P. falciparum*. *In vitro* culture was done by candle jar method[10]. Parasitized blood (1.5 ml) was collected aseptically from a patient admitted to Cant. Hospital, Lucknow, in 0.5 ml of ACD (acid citrate dextrose solution) and mixed gently. The blood sample was immediately transported to laboratory on wet ice and washed twice with RPMI-1640. An 8% suspension of washed cells was then made in RPMI complete medium with 10% AB human serum[11]. This suspension (1.5 ml) was then placed in flat-bottomed glass vials (22 × 55 mm) and the vials kept at 37°C in a candle jar. The medium was changed every 24 h and the parasite growth was monitored by counting the Giemsa stained thin smears daily.

During the first two weeks the parasitaemia varied between 0.1 and 0.2% and then started rising and reached about 4% on 26th day. The parasites were maintained in culture up to 3 months by subculturing with A^+ red cells every 4 or 5 day. At this stage a few vials of culture were cryopreserved.

In order to test the chloroquine sensitivity of this Indian isolate (DCK-l), three standard isolates (Z, KZI and Ituxi 084; obtained from NIH, Bethesda, USA) having different chloroquine sensitivities, were also maintained in *in vitro* culture for one month and used as reference isolates for chloroquine sensitivity test. Among these isolates, Z is sensitive, KZI is mildly resistant and Ituxi 084 is highly resistant to chloroquine[12].

A micro *in vitro* test[13] was standardized and used for determining the chloroquine sensitivity. Briefly, 4-day-old culture of all the four isolates (DCK-1, Z, KZI and Ituxi 084) were synchronized by treating them with 5% sorbitol[14], washed once with RPMI-1640, resuspended and mixed with washed normal erythrocytes to get 50% erythrocyte suspension in complete growth medium (with approximately 1% parasitaemia). The test was performed in 96 well polystyrene plates predosed with graded concentrations of chloroquine (0.05 p mol to 32 p mol). Each well received 10 μl of 50% erythrocyte suspension (1% parasitaemia) and 100 μl of complete RPMI medium. Triplicate wells for different concentrations of chloroquine and four wells for controls were used for each of these isolates. The plates were transferred to a candle jar and kept at 37°. After 30–40 h of incubation thin smears from each well were prepared and Giemsa stained. Mean schizont counts per 100 parasites were determined for controls as well as for each drug concentration. All concentrations of chloroquine were expressed as p mol/μl of blood in culture.

Results of chloroquine sensitivity for all the four isolates are given in table 1. Inhibitory chloroquine concentration for DCK-1 isolate is 0.494 ± 0.003 p mol/μl of blood while for Z, KZI and Ituxi 084 isolates the inhibitory concentrations are 0.923 ± 0.024, 10.208 ± 0.208 and 26.597 ± 0.524 p mol/μl of blood respectively.

The *in vitro* inhibitory concentrations of chloroquine for DCK-1 and Z isolates are within the range of 1 p mol/μl blood as reported earlier for the chloroquine sensitive ioslates of *P. falciparum*[15]. Another sensitive isolate (FAN-5) has an inhibitory concentration of 0.5 p mol/μl blood of chloroquine[7]. Therefore, on the basis of these results, it appears that DCK-1 (new Indian isolate) is a chloroquine sensitive isolate. Further work on biochemical and immunochemical characterization of this isolate as well as its comparison to other Indian isolates of *P. falciparum* is in progress.

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REDIFFERENTIATION OF NaCl TOLERANT SUGARCANE PLANTS FROM CALLUS DERIVED RESISTANT LINES

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SALT-resistant cell lines have already been selected in *Nicotiana sylvistris*[1], *N. tabaccum*[2], *Medicago sativa*[3], *Citrus sinensis*[4] and in some grain legumes[5]. However, in most cases regeneration of plants from resistant cell lines is difficult to achieve[6]. The selection of a sugarcane cell line tolerant to NaCl was earlier accomplished, but many problems in recovering an entire plant have been discussed[7]. The present communication reports a successful attempt of regenerated salt tolerant sugarcane plants from varieties Co740 and Co62175, those were mainly derived *in vitro* from the callus cells grown in high salt conditions. Salinity is an increasing problem in the sugarcane fields of India and these two cultivars are salt-sensitive[8]. Hence the selection and regeneration of a salt-resistant plants are of great importance.

The callus was raised from stem tip and young leaves on modified Murashige and Skoog[9] (MS) medium as suggested by Heinz and Mea[10]. The actively growing callus was subcultured at 15–30 day intervals.

For selection of salinity tolerant line the salt (NaCl) was added into the basal MS medium during subculturing of callus on agar medium. Salt concentrations at various levels starting from 0.1, 0.2, 0.5, 1.0, 1.5 and 2.0% were used.

The relative growth response of the callus cultures of sugarcane var. Co62175 and Co740 on MS medium with increasing concentration of NaCl is shown in figures 1–3. It can be seen that both varieties showed stimulation of the growth up to 0.2% of NaCl in basal MS medium, while the growth was inhibited beyond 0.5% NaCl and at about 1% the growth was completely inhibited. At this concentration the callus shows browning, compactness and subsequent death of the tissue. Nevertheless, small sectors of good growing tolerant cells were observed in some culture tubes even at a higher NaCl level (1.5%). These actively growing cell aggregates were then isolated and subcultured

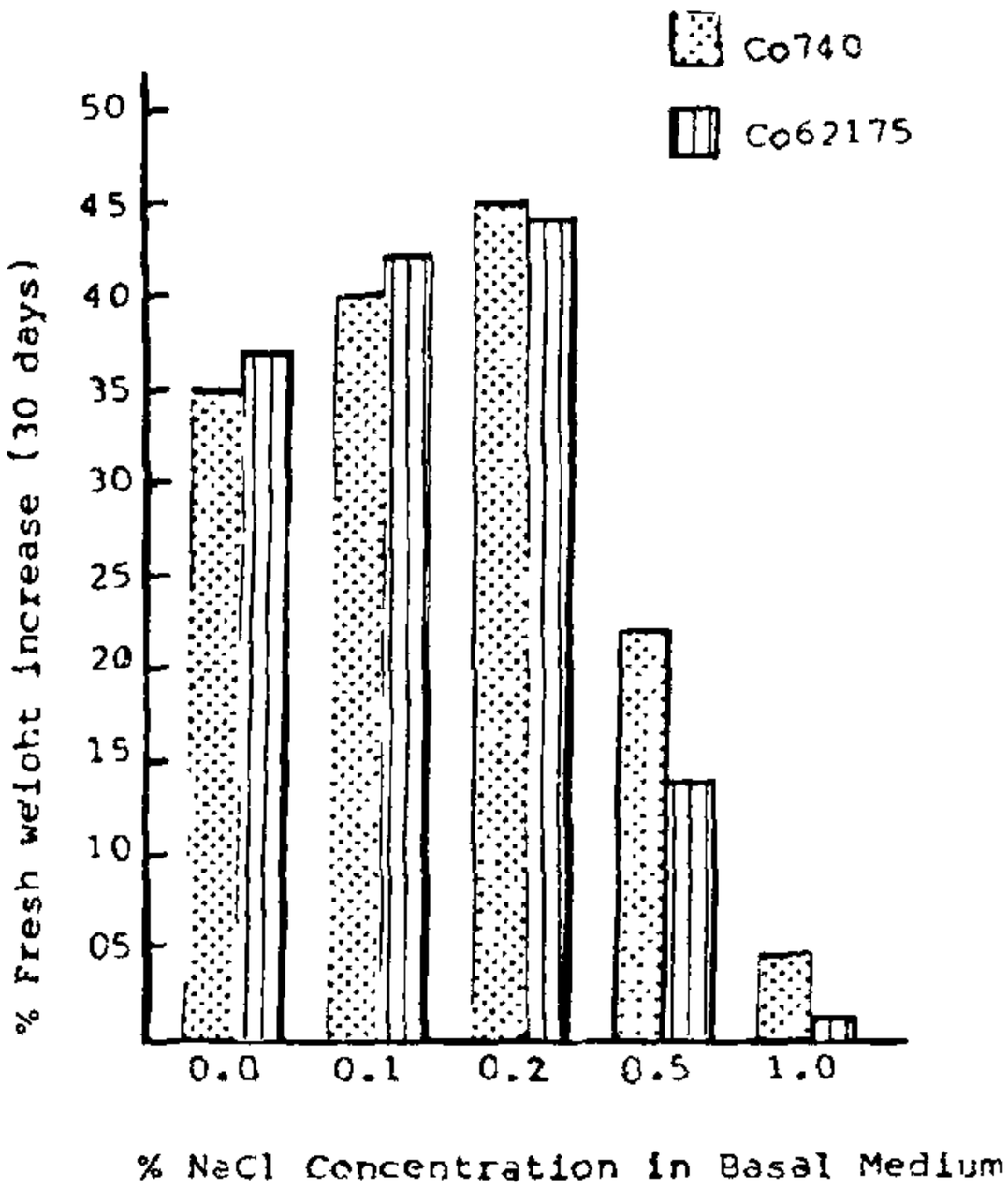

Figure 1. Growth of sugarcane callus in different salt concentrations.