Table 1 *Separation of mycobacillin from co-purified mixture of mycobacillin and comycobacillin*

	Co-purified mixture[1]	DEAE-cellulose
R_f values of the constituent peptides[2]		
1. Mycobacillin	0.70	0.70
2. Comycobacillin	0.60	ND
Content of the constituent peptide (mg/mg peptide)[3]		
1. Mycobacillin	0.65	1.00
2. Comycobacillin	0.35	ND
Yield of purified mycobacillin	—	92%

1. Prepared according to Majumder and Bose[4].
2. On the thin layer chromatogram developed with the solvent system *n*-propanol—25% ammonia (2:1 v/v).
3. Comycobacillin has no antifungal activity but it stimulates the antifungal action of mycobacillin (unpublished) so the antibiotic content is measured in terms of serine (present only in mycobacillin) in the acid hydrolysate of the mixture.
ND = Not detectable.

then centrifuged and the supernatant was charged to the top of the column (4 × 1 cm). The column was then eluted with benzene-methanol-acetic acid (glacial) (85.5:9.5:5.0 v/v). The eluent was collected in 0.5 ml fractions which were monitored by measuring absorbance at 280 nm (figure 1). The positive fractions were pooled and evaporated under reduced pressure. Sometimes it was necessary to dissolve it in the minimum volume of ethanol and then to reprecipitate it, by adding ten volumes of cold (0°C) ether.

The purified antibiotic was free from comycobacillin. About 6 mg of the pure antibiotic was thus obtained (table 1). The method was convenient both in respect of time and labour. Regarding the mechanism of purification it is felt that the antibiotic, being a polar peptide containing a number of aspartic acid and glutamic acid residues[6], benzene and methanol have created an optimum condition just enough to retain it, in the column along with comycobacillin (pure cellulose does not retain the peptide mixture under identical conditions). The subsequent use of acetic acid could ensure selective elution of the antibiotic from its association with comycobacillin. Thus this method would be useful for the purification of a polar peptide on DEAE-cellulose column.

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ENZYMES OF NITROGEN ASSIMILATION IN DEVELOPING ENDOSPERM OF NORMAL AND OPAQUE-2 MAIZE (*ZEA MAYS* L)

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THE average absolute activities (per endosperm) of nitrogen assimilatory enzymes, glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate dehydrgenase (GDH), and glutamate oxaloacetate transaminase (GOT) were substantially higher in developing opaque-2 maize endosperm as compared to normal maize endosperm. The activity of nitrate reductase (NR) was, however, very low both in normal and in opaque-2 developing endosperm. The present study suggests that low protein content in opaque-2 maize endosperm at maturity is not due to limitation of the key enzymes of nitrogen assimilation.

It is generally recognized that the improvement of nutritional quality in opaque-2 maize is due to the suppressed synthesis of major storage protein zein[1-3] which is extremely deficient in lysine. It is not known as to why the net reduction in protein content in endosperm occurs. It has been speculated that the increase in RNase activity results in degradation of mRNA towards the later stages of endosperm development and consequently protein synthesis is reduced[4-6]. In addition to the regulation of mRNA synthesis, the levels of different amino acids could also play an

important role in regulating the net amount of protein synthesized in the grain. GOGAT and GS have been shown to be the major enzymes of ammonia assimilation in plants and under conditions of high ammonia level GDH may also play a part[7]. Therefore, in the present study, changes in NR, GS, GOGAT, GDH and GOT during development have been studied with a view to ascertaining if there are any limitations in ammonia assimilation and consequently their role in storage protein biosynthesis.

A normal maize inbred line Fla 3H94 and its opaque-2 isogenic line were grown at the farm of IARI, New Delhi during monsoon season. Individual ears were self-pollinated. The ears were harvested at 15, 20, 25 and 30 days after pollination and the endosperms were collected after removing the pericarp and the embryo from the kernels at 4°C. The endosperms were hand-ground using liquid nitrogen in a mortar and pestle and then with extraction buffer 0.2 M Tris-Cl, pH 7.5 (1 : 2 W/V). The homogenate was centrifuged at 15000 g for 20 min and the supernatant was used as crude enzyme for studying the activities of GS, GOGAT, GDH and GOT. All the operations were carried out at 0–4°C. Synthetic activity of GS was assayed by the procedure of Kanamori and Matsumoto[8], while GOGAT activity was assayed spectrophotometrically at 30°C by following the oxidation of NADH at 340 nm according to Sodek and Dasilva[9]. GDH was also assayed spectrophotometrically at 37°C following the oxidation of NADH according to Bulen[10]. Corrections were made for substrate-independent NADH oxidation.

GOT activity was assayed spectrophotometrically at 37°C as described by Gupta *et al.*[11]. *In vivo* assay of NR was carried out according to Klepper *et al.*[12]. The activities have been expressed in absolute terms on per endosperm basis.

The values reported are an average of two experiments each carried out in duplicate.

Nitrate reductase (NR) activity was quite low in both normal and opaque-2 developing endosperm ranging from 0.4 to 1.4 n mol NO_2 formed per 30 min per endosperm. There was limited variation in the activity between normal and opaque-2 endosperm at all the stages. The activity of GS per endosperm initially increased from 15-day to 20-day stage in both normal and opaque-2 maize and decreased thereafter at 25 and 30 day stages in normal maize (figure 1A). In opaque-2 maize endosperm it decreased upto 25th day and did not change much thereafter. At day 15, the activity was higher (1.9 fold) while at days 20 and 30 it was lower in the normal as compared with opaque-2 maize endosperm. GOGAT activity per endosperm in normal maize increased upto day 25 and decreased thereafter at day 30. In opaque-2 endosperms the activity increased substantially at day 20 and did not change much thereafter (figure 1B). The activities in both normal and opaque-2 endosperms were more or less comparable at 15 and 25 day stages while at 20 and 30 day stages the activities were significantly higher (about 2 fold) in opaque-2 as compared to normal maize endosperms. GDH activity per endosperm in normal maize increased gradually upto day 25 and decreased at day 30 while in opaque-2 endosperm it increased upto day 20, remained same at 25-day stage and again increased substantially at 30-day stage (figure 1C). The activities were more or less the same in normal and opaque-2 at 15- and 25-day stages, while at 20 and 30 day stages the activities were respectively 1.4 and 2.2 fold higher in opaque-2 as compared to normal maize endosperm. GOT activity per endosperm in normal and opaque-2 maize endosperm during de-

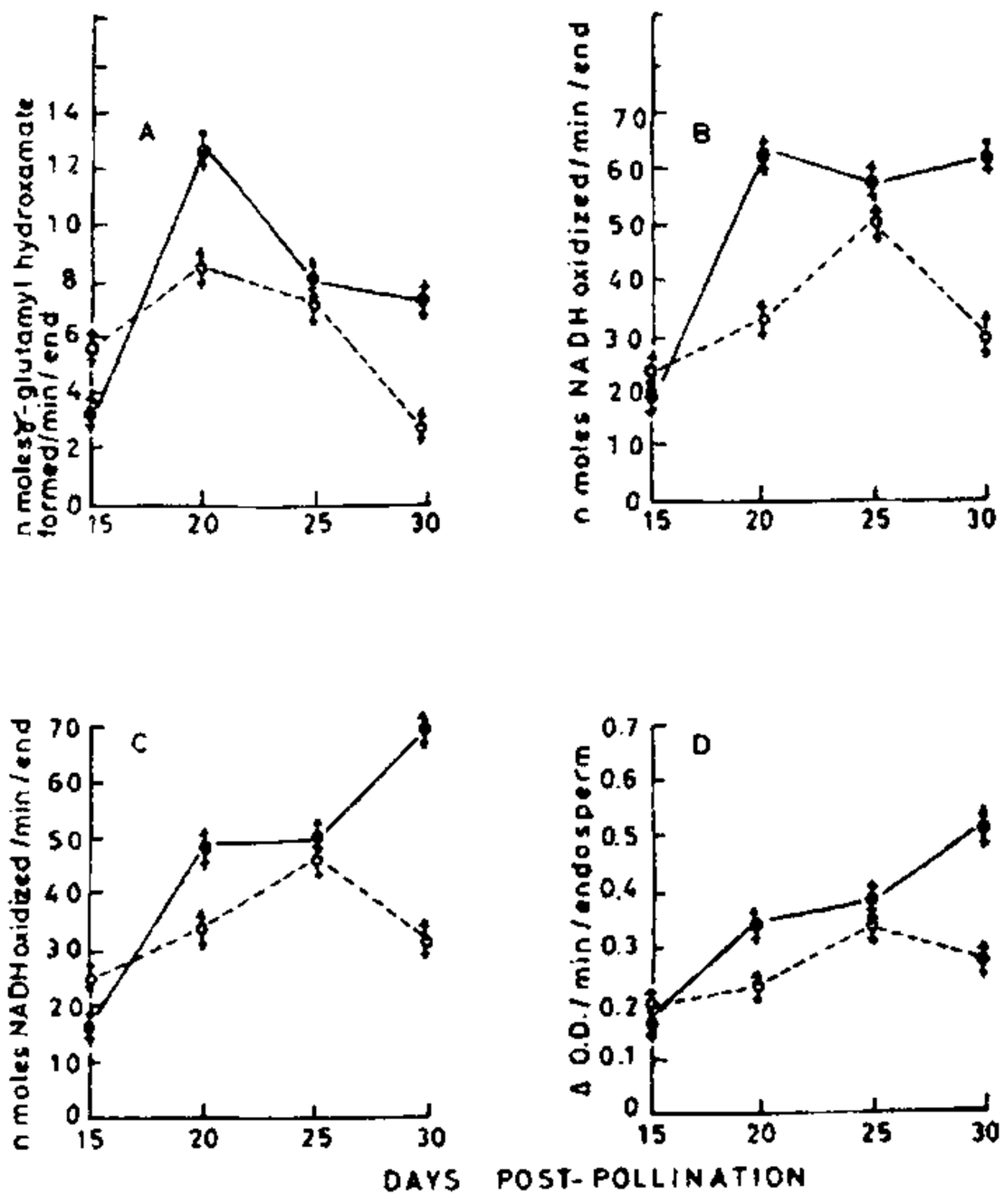

Figure 1. Changes in absolute activities (per endosperm) of nitrogen assimilatory enzymes, glutamine synthetase (A), glutamate synthase (B), glutamate dehydrogenase (C), and glutamate oxaloacetate transaminase (D) in developing endosperm of normal (o - - - - o) and opaque-2 (●————●) maize.

velopment followed a trend similar to that observed for GDH. The activities were nearly comparable in normal and opaque-2 maize endosperms at 15 and 25 day stages, while at 20- and 30- day stages the activities were respectively 1.5 and 1.8 fold higher in opaque-2 as compared to normal endosperm (figure 1D).

Maize plants transport nitrogenous compounds primarily in the form of glutamine[13] and evidence from tracer feeding suggests that developing wheat kernel[14,15] and maize endosperms[16,17] are capable of synthesizing many of the amino acids required for the formation of their storage proteins. Glutamine may account for about 25% of total amino acids in these proteins[3,18]. Other amino acids are presumably supplied as a result of protein turnover in the leaves[19] or they are synthesized in the endosperm. GOGAT is a key enzyme of ammonia assimilation pathway and results in the synthesis of glutamate. The glutamate so formed functions as a donor for α-amino groups[7] in transamination reactions resulting in the synthesis of other amino acids.

Opaque-2 maize has been shown to contain less protein per endosperm[20,21] and per kernel[22] at maturity than normal maize. This is mainly because of reduced zein synthesis in the presence of opaque-2 genes; opaque-2 maize protein contains 15 to 27% zein as against 54 to 59% in normal maize endosperm[23,24]. From the present study it appears that the nitrate is not a major nitrogen source in developing normal and opaque-2 maize endosperms as nitrate reductase activity is just negligible. GS and GOGAT are present at fairly high levels in both normal and opaque-2 developing endosperms and probably represent the major pathway for assimilation of ammonia. Sodek and Dasilva[9] have shown that GOGAT is an important enzyme in the maize endosperm. The average activities of all the key nitrogen assimilatory enzymes *e.g.* GS, GOGAT, GDH and GOT in opaque-2 maize endosperms are higher than normal maize endosperms during development. Thus it can be suggested that lower rate of protein synthesis, in general, and that of zein synthesis, in particular, in opaque-2 maize endosperm is not due to the limitation of nitrogen assimilatory enzymes. Higher level of these enzymes in opaque-2 endosperms may be contributing to higher free amino acid pool[25].

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