Table 2 *Percentage of reversion to parental phenotype in early segregating generations of triticale × bread wheat crosses*

Cross	Generation	No. of plants observed	Triticale type (%)	Intermediate type (%)	Wheat type (%)
UPT 72142 × UP 262	F_2	40	33	23	45
	B_1	25	52	20	28
	B_2	25	60	8	32
UPT 72142 × HD 2009	F_2	40	45	18	38
	B_1	25	60	28	12
	B_2	25	36	8	56
UPT 75233 × HD 2009	F_2	40	25	40	35
	B_1	25	56	32	12
	B_2	25	28	20	33
UPT 78267 × UP 262	F_2	40	50	13	38
	B_1	25	72	20	8
	B_2	25	76	12	12
PR 673 × UP 262	F_2	40	33	18	25
	B_1	25	32	20	48
	B_2	25	28	32	40

fertilization[3]. The primary hexaploid triticale swhich have a genomic constitution AABBRR are likely to carry these genes since, they are located on A and B genomes only and rye genotype has no influence on crossability[4]. It shows that the genotype of the female parent is important in controlling the crossability.

The percentage of reversion to the parental types is given in table 2. Plants in the segregating generations were classified into triticale, wheat and intermediate types. Those with compact spike and prominent awns were classfied as triticale, type plants with lax spike and comparatively short awns were classified as wheat type. Other plants with mixed morphological features of wheat and triticale were classified as intermediate type. Useful transgressive segregants were observed in all the generations.

It is suggested that the desirable segregants of triticale and intermediate type in the F_2 generation of triticale × wheat crosses may be further mated *inter se* in order to enlarge the genetic variability.

Part of the thesis submitted by the Senior author in partial fulfilment of the degree of Master of Science in Agriculture (Plant Breeding) of G. B. Pant University of Agriculture and Technology, Pantnagar.

5 May 1984; Revised 13 August 1984

1. Lein, A. Z., *Ind. Abst. Vererbugal*, 1943, **81**, 28.
2. Riley, R. and Chapman, V., *Genet. Res. Camb.*, 1967, **9**, 259.
3. Lange, W. and Wojciechowska, B., *Euphytica*, 1976, **25**, 609.
4. Krolow, K. D., *Z. Pflanzenzuecht*, 1964, **51**, 21.

INVOLVEMENT OF CALCIUM IN NITROGEN FIXATION BY *NOSTOC LINCKIA*

D. K. MISHRA, M. JHA and H. D. KUMAR
Centre of Advanced Study in Botany,
Banaras Hindu University, Varanasi 221005, India.

CULTURES of *Nostoc linckia* rapidly and significantly but not completely, lost their ability to reduce acetylene when incubated with 2 mM of ethyleneglycol-bis- (B-aminoethyl ether)-N,N,N′-tetra-acetic acid (EGTA) in light. The alga resumed diazotrophy when supplied with 4 mM of calcium chloride. It is suggested that EGTA might deplete the calcium ions from the cyanobacterial cells, and thereby destroy a calcium-dependent process by which nitrogenase is protected from inactivation by oxygen.

Cyanobacterial nitrogen fixation is carried out by unicellular, filamentous heterocystous and filamentous non-heterocystous forms[1,2]. Diazotrophic mechanism requires a strong reductant and ATP for the production of ammonia. Heterocystous cyanobacteria have developed an exchange system between hetero-

cysts and vegetative cells. Heterocysts export fixed nitrogen in the form of glutamine to vegetative cells and subsequently receive aerobically fixed CO_2 and an unidentified compound(s)[3].

Nitrogen fixation in several species of cyanobacteria is repressed by the addition of chelating agents[4]. The addition of ethyleneglycol-bis-(B-aminoethyl ether)-N,N,N′-tetraacetic acid (EGTA) which binds Mg^{++} weakly but binds Ca^{++} strongly into a medium would decrease the concentration of free divalent cations in the medium, causing their efflux from the cyanobacterial cells. This might then inhibit nitrogenase activity indirectly. This paper describes investigations on the nature of the Ca^{++} requirement for nitrogen fixation in the heterocystous cyanobacterium, *Nostoc linckia*.

Nostoc linckia was grown axenically in Allen and Arnon's[5] medium adjusted to pH 7.5 after autoclaving. All growth and cultural conditions were the same as described earlier[6].

The acetylene reduction assay of Stewart *et al*[7] was used to measure nitrogenase activity. Assay was performed in calibrated serum bottles of about 8.0 ml capacity. Samples (2.0 ml each) of exponentially growing cultures were placed in triplicate bottles and sealed with serum stoppers. The partial pressure of acetylene was kept at 0.1 atm. Reactions were run for 30 min at 28°C and 3000 lux. The ethylene produced in the reaction vessel was estimated in a CIS gas chromatograph (Baroda) fitted with a Porapak R column and a hydrogen flame ionization detector. Nitrogen was used as the carrier gas.

On addition of EGTA to illuminated aerobically grown nitrogen fixing cultures, nitrogenase activity was rapidly inhibited by 80% within 2 hr (figure 1). Following a 2 hr exposure to EGTA, no increase in nitrogenase was recorded within 10 hr of resuspension in fresh medium. On the other hand, addition of 4 mM-$CaCl_2$ within 30 min, following the addition of 2 mM-EGTA almost completely prevented any inhibition of acetylene reduction and within 2 hr nitrogenase activity reached almost the same level as that of control cultures.

All nitrogenases when isolated are sensitive to oxygen. The chemical reactions which lead to damage of both nitrogenase proteins by oxygen have not been elucidated. Oxygen may produce superoxide radicals or H_2O_2 which destroy the enzyme. A particular role in protecting nitrogenase from oxygen is often attributed to heterocysts. Since heterocysts are unable to evolve oxygen photosynthetically, nitrogenase inside these cells must not be protected against this extra gas formation. However, heterocysts do respire and oxidative phosphorylation can support nitrogen fixation in dark respiration. Therefore, oxygen can reach the inside of heterocysts and nitrogenase must be protected therein as in other aerobes. Oxygen may pass through microplasmodesmata connecting heterocysts and vegetative cells.

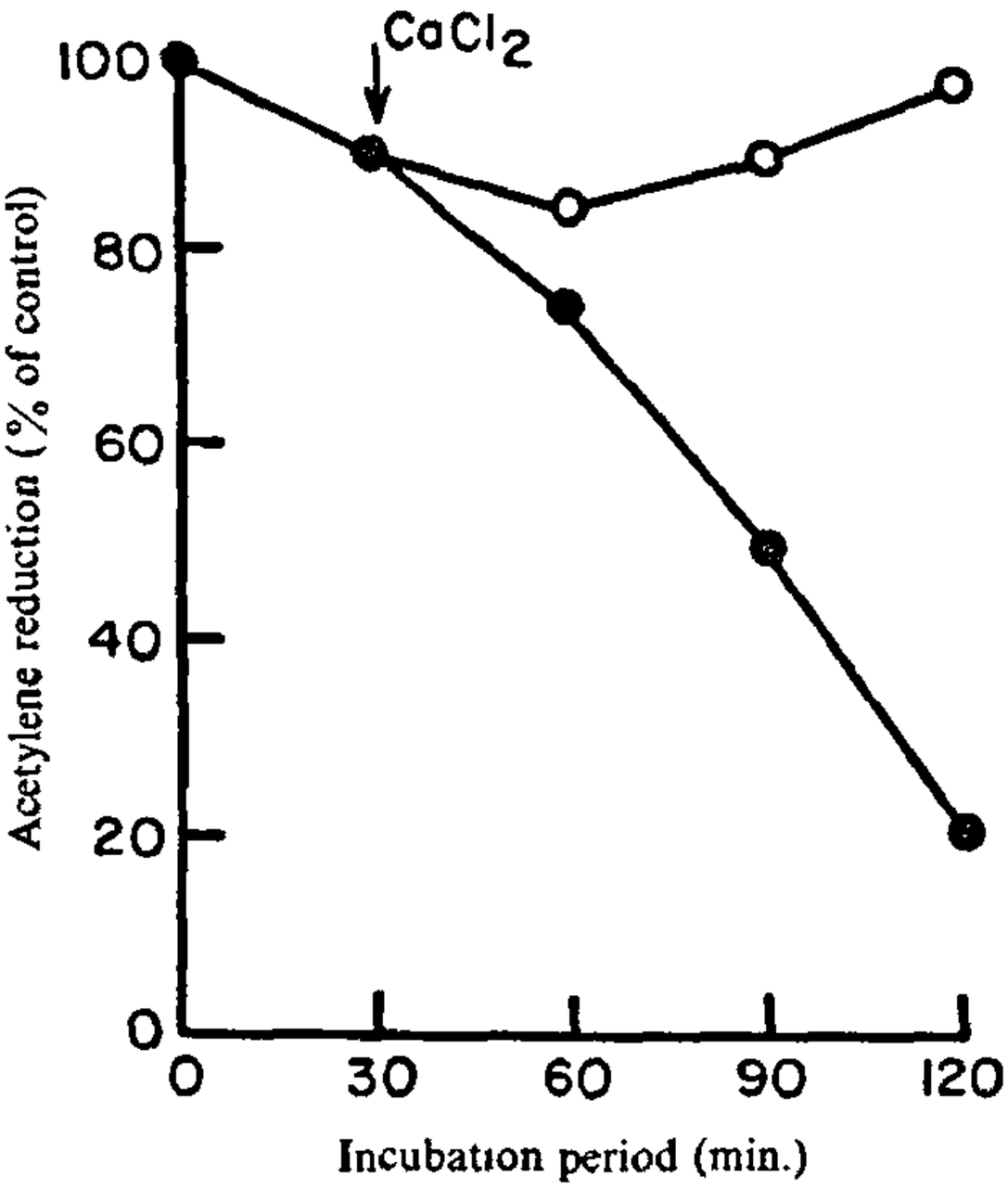


Figure 1. Effect of 2 mM-EGTA (added at the time of initiation of experiment) and 4-mM $CaCl_2$ (added after 30 min of initiation of experiment) addition on nitrogenase activity of aerobically grown nitrogen-fixing cultures of *Nostoc linckia*. Activity is expressed relative to the activity of a control to which no EGTA was added. Each point represents the mean of at least three determinants.

Two possible explanations for the effect of EGTA on nitrogenase activity were examined. First, EGTA may inhibit Ca^{++}-stimulated ATPase activity, inhibiting nitrogenase activity. Secondly, addition of EGTA to cultures of *Nostoc linckia* might disrupt a Ca^{++}-dependent mechanism which normally protects nitrogenase from inhibition by oxygen. Because Ca^{++} in association with superoxide dismutase and catalase is particularly associated with nitrogenase within heterocysts, it prevents toxic effects of superoxide[8].

The authors (DK and MJ) thank the University Grants Commission for financial assistance and the

Head, Department of Botany, B.H.U., Varanasi for facilities.

19 September 1984

1. Hamadi, A. F. and Gallon, J. R., *J. Gen. Microbiol.*, 1981, **125,** 391.
2. Bothe, H., Neuer, G., Kalbe, I. and Eisbrenner, G., *Proc. Int. Symp. on Nitrogen Fixation* (eds) W. D. P. Stewart and J. R. Gallon, Academic Press, New York, 1980, p. 83.
3. Thomas, J., Meeks, J. C., Wolk, C. P., Shaffer, P. W., Austin, S. M. and Chein, W. S., *J. Bacteriol.*, 1977, **129,** 2138.
4. Gallon, J. R., *Ecol. Bulletin*, 1978, **26,** 20.
5. Allen, M. B. and Arnon, D. I., *Plant Physiol.*, 1955, **30,** 366.
6. Kumar, H. D., Mishra, D. K. and Tripathi, A. K., *Plant and Nature*, 1983, **1,** 1.
7. Stewart, W. D. P., Fitzgerald, G. P. and Burris, R. H., *Arch. Microbiol.*, 1968, **62,** 336.
8. Henry, L. E. A., Gogotov, I. N. and Hall, D. O., *Biochem. J.*, 1978, **174,** 373.

CHROMOSOME NUMBER AND KARYOTYPIC STUDIES IN *GLYCYRRHIZA*

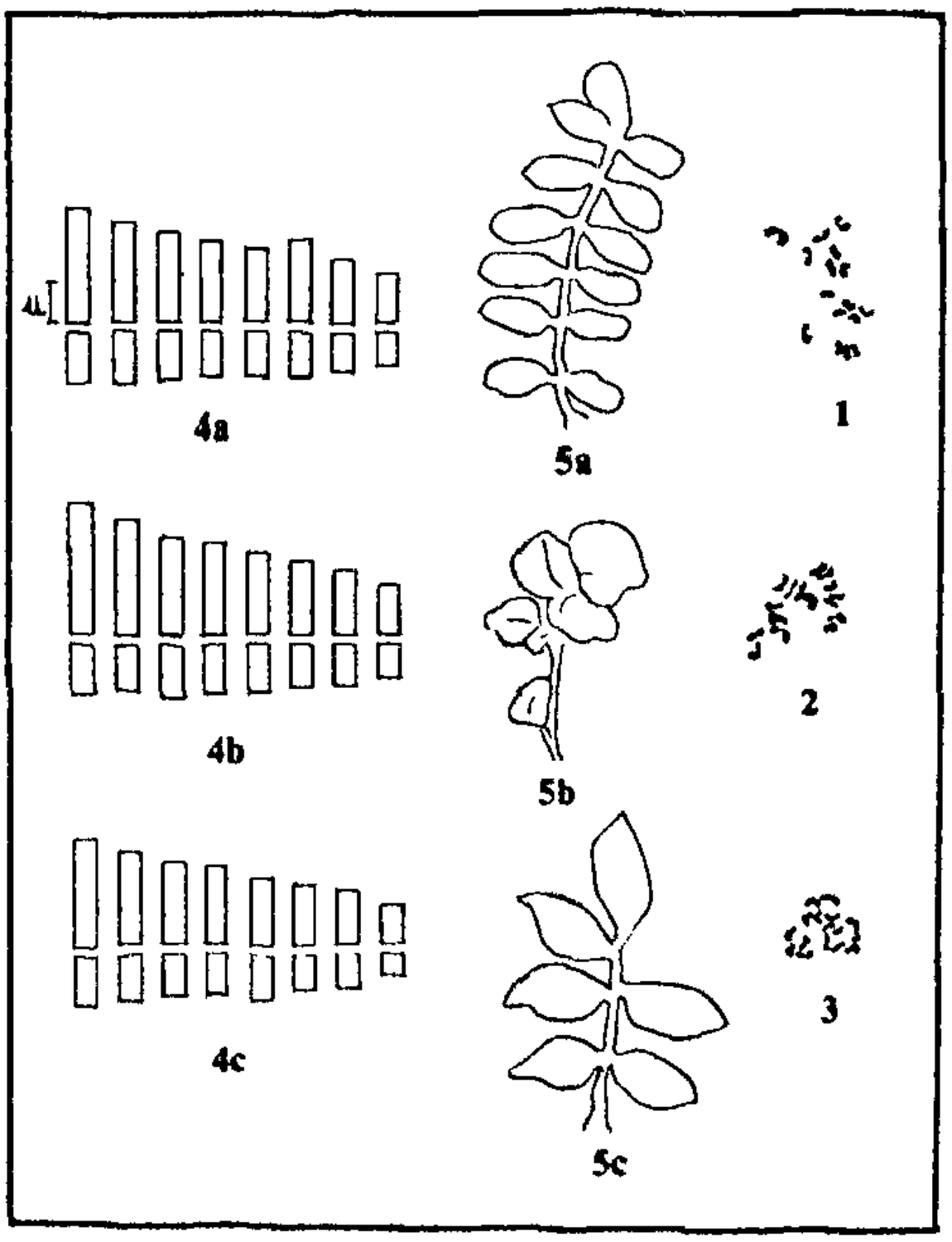
SHEELA VERMA* and R. S. NADKARNI
Department of Botany, Institute of Science, Nagpur 440 010, India.
**Present address: Medicinal and Aromatic Plants Research Unit, College of Agriculture, Indore 452001, India.*

GLYCYRRHIZA is a genus of perennial herbs and undershrubs distributed in the subtropical and warm temperate regions of the world. This genus is of great medicinal importance as glycyrrhizic acid and various alkaloids extracted from different species are used in different types of medicinal preparations mainly in cough syrups. This genus is commonly known as liquorice as it yields the product of commercial importance. So far, no species of *Glycyrrhiza* has been reported from India but cultivation of *G. glabra* and some other species has been undertaken at several places in India. Though the genus is of great medicinal value, previous reports indicate that very little cytogenetic work has been done. All the available papers deal only with chromosome numbers[1-5].

The present work is the account of chromosome number and karyotypic studies in three species *viz. Glycyrrhiza glabra, G. macdonica* and *G. uralensis*. The chromosome number and karyotype of *G. macdonica* are reported for the first time.

The seeds and rhizome cuttings of *G. glabra, G. macdonica* and *G. uralensis* were obtained from Indian Council of Agricultural Research Co-ordinated Project on Medicinal and Aromatic Plants, New Delhi. The chromosome study was made from healthy root tips using 8-hydroxyquinoline for pre-treatment and haematoxylin as stain. For studying the details of morphological characters of chromosomes, forty metaphase plates were selected, and for centromeric position, the standardized method of Levan *et al*[6] was followed.

The basic number in all the three species is $2n = 16$ (figures 1–3). The measurements and other details of the somatic chromosome in *G. glabra, G. uralensis* and *G. macdonica* are presented in table 1. The chromosomes in these three species are idiogrammed in figure



Figures 1–5. Chromosome number and karyotypic studies of *Glycyrrhiza glabra, G. macdonica* and *G. uralensis*. **1–3.** Basic numbers, **4a–c.** Idiograms of chromosomes, **5a–c.** Line diagrams of leaf morphology.