APPENDIX

ESTIMATION OF DNA OF SOME TAXA INVESTIGATED

During the past several years, abundant data have been obtained regarding deoxyribonucleic acid (DNA) content per nucleus and per chromosome among plant species (Sato, 1963; Rothfels et al., 1966; Rees and Jones, 1967, 1972; Rothfels and Heimberger, 1968; Jones and Rees, 1968; Rees, 1972; Bennett, 1972; EL-Lakany, 1972; EL-Lakany and Dugle, 1972; Sparrow et al., 1972; SZ-Borsos, 1973; Nagl et al., 1973; Grant, 1969, 1976). Most of these works have been reviewed recently by Price (1976). A correlation of DNA content with ploidy level and total chromatin matter made suggest that the DNA content per nucleus of somatic cell is constant in each species (Sato, 1963). This supports the so-called DNA-constant hypothesis. Thus there is some relationship between chromosome number and DNA content per nucleus. However, the extensive variation in DNA content among angiospermic families and among genera and species of the families are known. Such observations have been made by Rees and Jones (1967), Bennett (1972), Sparrow and Neumann (1974) in the family Poaceae and by Rees and Hazarika (1967), Martin (1968), Chooi (1971), Bennett (1972) in the family Leguminosae.

Though there is no overall correlation between chromosome

size or nuclear DNA content and phylogeny in angiosperms (Stebbins, 1938, 1950, 1966), the distribution of DNA content is very specific. Studies of closely related plant species indicate that both evolutionary increase and decrease in DNA content is apparently common. Rees and Hazarika (1967) based on determination of the nuclear DNA content of eighteen diploid (2n = 18) species of Lathyrus have proposed that, diminution of DNA content must have occured during evolution of species. Similarly Nagl et al.,(1973) while working with family Asteraceae have shown that, the phylogenetic reduction in chromosome size is closely related with reduced DNA-content of a diploid nucleus.

In the present study an attempt is made to see whether a similar correlation (i.e. correlation of reduced DNA-content with phylogenetic reduction) exists in the taxa investigated. Totally 20 species belonging to the genera Indigofera, Desmodium and Alysicarpus of Fabaceae are selected for the present work.

MATERIALS AND METHODS :

Seedlings at open cotyledon stage (7 days old) were taken for the estimation. The different species for which estimation is carried out are listed in Table 1.

Preparation of extract :

A 20% homogenate of the radicle free seedlings was prepared by grinding in pestle and morter for 20 min. in methanol at room temperature. This homogenate was used for the estimation of DNA following Schneider's method (1957).

Estimation of DNA :

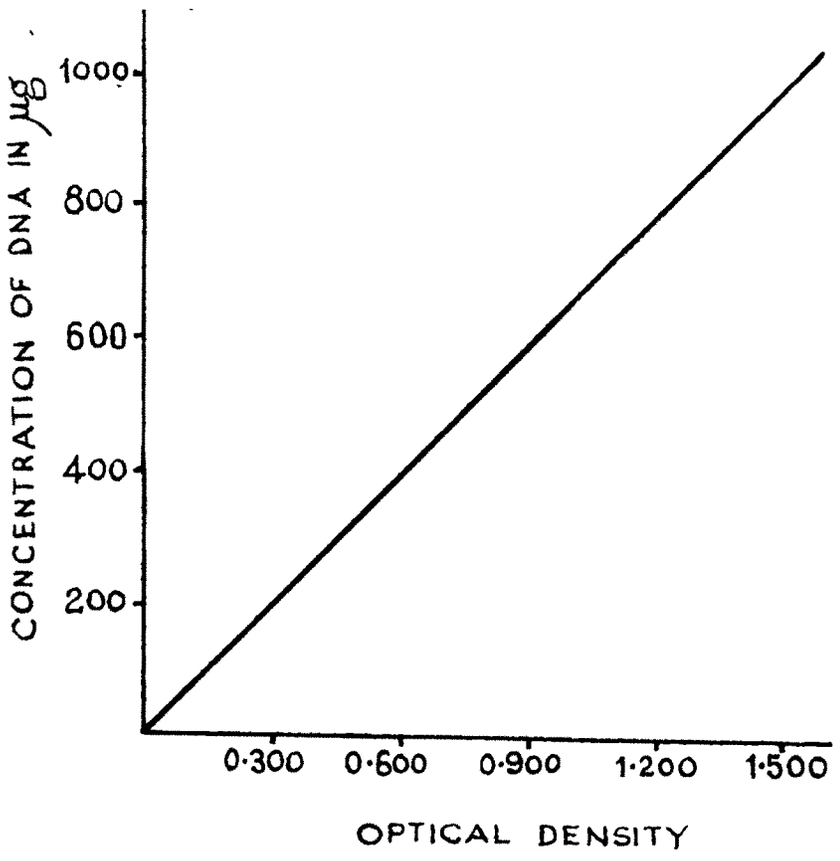
The determination of DNA is based on its preferential solubility in hot trichloro-acetic acid (TCA), which is quantitated by means of colorimetric reactions involving the pentose component of DNA.

5 ml of homogenate was centrifuged at 1500 x g for 10 min. and the residue obtained was made pigment free by giving 5-6 washes following the method of Smille and Krotkov (1960). The residue was then suspended in 2 ml of 5% TCA and allowed to stand for 15 min. at room temperature. This was then centrifuged for 15 min. at 1500 x g. The residue obtained was suspended in 5 ml of 95% ethanol, kept in boiling water for 30 seconds, cooled and centrifuged at 1500 x g for 15 min. The residue obtained was suspended in 2 ml of 5% TCA, kept in hot water both at 90°C for 15 min. by occasional stirring. This was cooled and centrifuged at 1500 x g for 15 min., the supernatant was collected. The residue was washed with

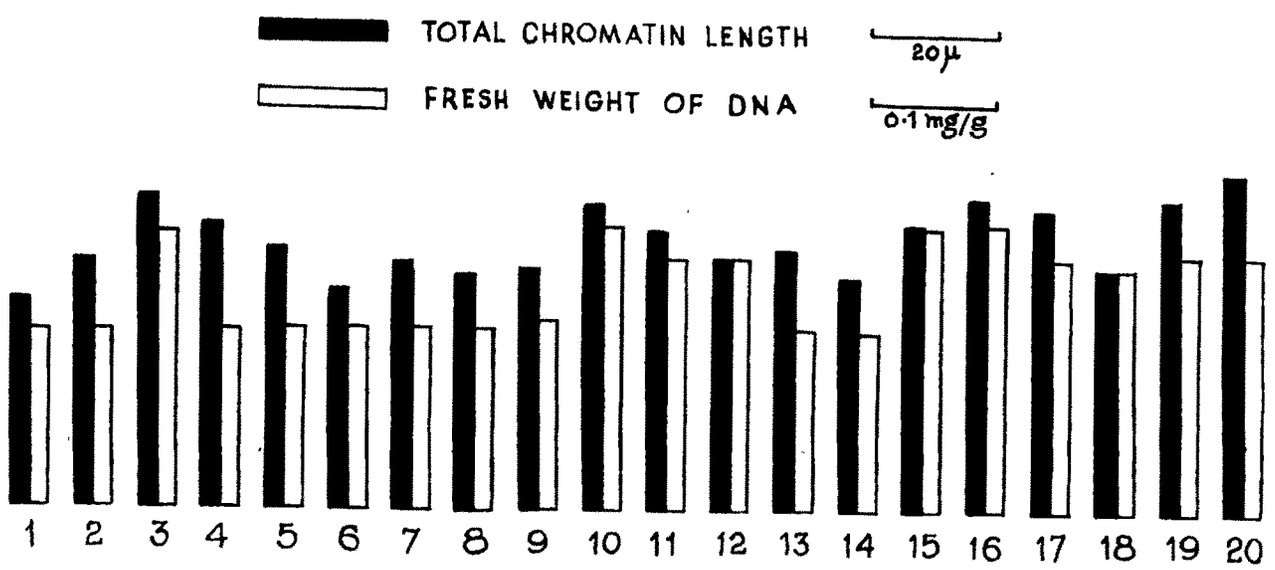
Fig. 1 A - Standard DNA graph.

Fig. 1 B - Comparision of total chromatin length with total DNA-content in :

1. Indigofera astragalina
2. I. hirsuta
3. I. trifoliata
4. I. linnaei
5. I. tinctoria
6. I. cordifolia
7. I.linifolia var.campbelli
8. I. linifolia
9. I. oblongifolia
10. I. glandulosa
11. Alysicarpus bupleurifolius
12. A. procumbens
13. A. longifolius
14. A. heyneanus
15. A. wallichii
16. A. styracifolius
17. A. monilifer
18. A. vaginalis
19. Desmodium dichotomum
20. D. gangeticum


CONCENTRATION OF DNA IN µg
1000
800
600
400
200
0·300
0·600
0·900
1·200
1·500
OPTICAL DENSITY

A


TOTAL CHROMATIN LENGTH
20µ
FRESH WEIGHT OF DNA
0·1 mg/g
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

B

2 ml 5% TCA and the supernatant obtained was added to the previous collection. This was used for DNA estimation using Diphenylamine reagent and the blue colour developed was read at 660 nm. using Carl Zeiss Spectrophotometer. Purified Calf Thymus DNA (BDH) was used as standard (Fig. 1A).

RESULTS AND DISCUSSION :

The results are summarised in the Table 1. As can be seen from the table that DNA concentration ranges from 0.044% to 0.061% by dry weight. Comparision of total DNA content with total chromatin length of the species investigated show good correlation (Fig. 1B). The increase in DNA content with the increase of total chromatin length is observed in majority of the species (Fig. 1B). Rees and Hazarika (1967) have shown, diminution of DNA content with the advancement of the species in the genus Lathyrus. Nagl et al., (1973) while, working with Asteraceae have also shown that, the phylogenetic reduction in chromosome size is closely related with reduced DNA content of diploid nucleii. The species of the genera Indigofera, Desmodium and Alysicarpus investigated presently do not show such correlation.

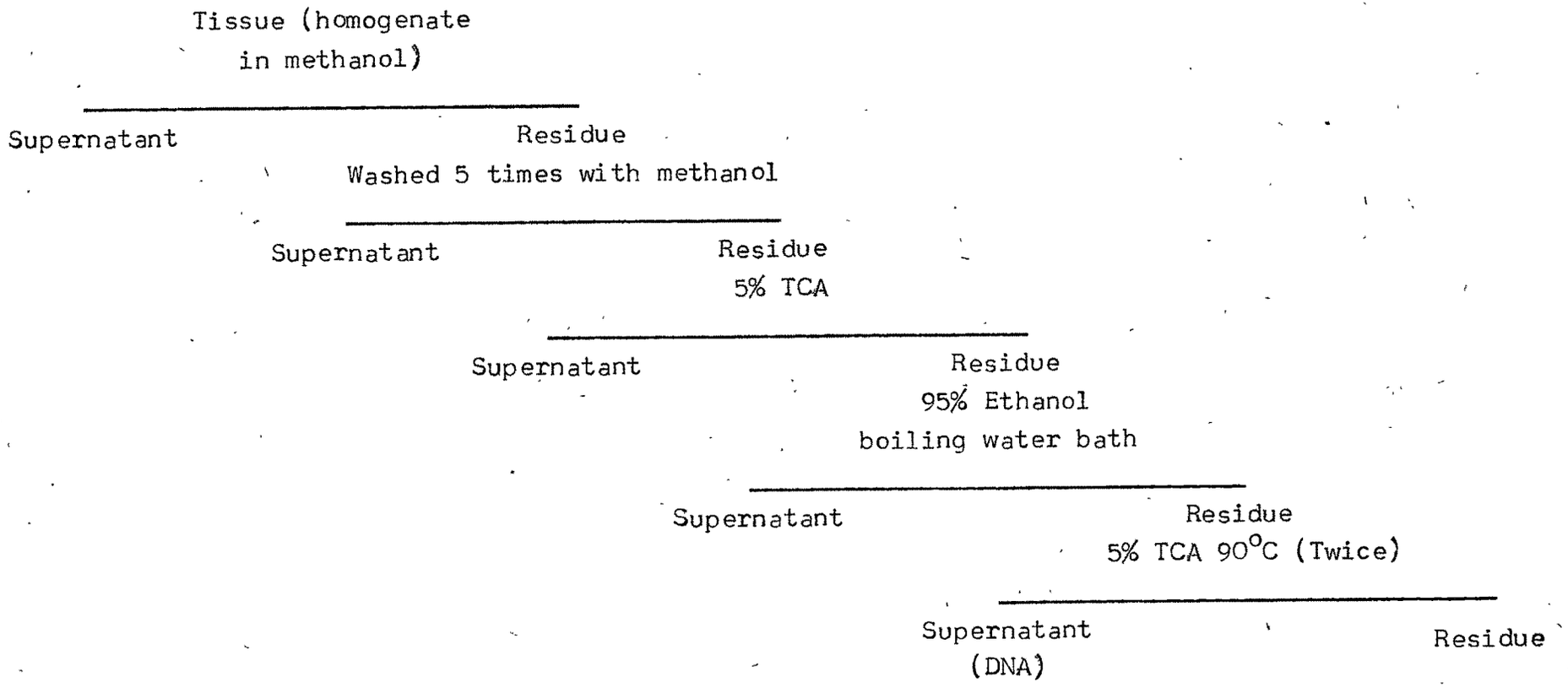

Tissue (homogenate
in methanol)
Supernatant
Residue
Washed 5 times with methanol
Supernatant
Residue
5% TCA
Supernatant
Residue
95% Ethanol
boiling water bath
Supernatant
Residue
5% TCA 90°C (Twice)
Supernatant
(DNA)
Residue

Table 1. Comparision of the total chromatin length with total DNA content of the taxa investigated.

Sr. No.	Taxa	Coll. No.	Somatic Number (2 n)	Total Chromatin length (in μ)	DNA	
					mg/g fresh wt.	g/100 g dry wt.
1.	*Indigofera astragalina*	6	16	32.56	0.182	0.044
2.	*I. hirsuta*	102	16	38.76	0.185	0.045
3.	*I. trifoliata*	30	16	48.58	0.233	0.057
4.	*I. linnaei*	23	16	44.14	0.182	0.044
5.	*I. tinctoria*	4	16	40.60	0.182	0.044
6.	*I. cordifolia*	25	16	34.42	0.185	0.045
7.	*I. linifolia* var. *campbelli*	18	16	38.66	0.182	0.044
8.	*I. linifolia*	19	16	36.78	0.185	0.045
9.	*I. oblongifolia*	11	16	37.10	0.195	0.048
10.	*I. glandulosa*	16	16	47.96	0.242	0.059
11.	*Alysicarpus bupleurifolius*	101	16	43.48	0.196	0.049
12.	*A. procumbens*	83	16	39.18	0.194	0.047
13.	*A. longifolius*	82	16	40.24	0.182	0.045
14.	*A. heyneanus*	34	16	36.20	0.185	0.045
15.	*A. wallichii*	89	16	44.52	0.242	0.061
16.	*A. styracifolius*	40	16	48.46	0.249	0.059
17.	*A. monilifer*	39	16	36.96	0.192	0.046
18.	*A. vaginalis*	66	16	36.96	0.182	0.044
19.	*Desmodium dichotomum*	50	22	48.30	0.209	0.050
20.	*D. gangeticum*	49	22	52.60	0.205	0.050