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A Comparative Karyological Study of Root and Embryo Tissue of a Few Genera of Leguminosae

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The family Leguminosae comprises of about 690 genera and more than 17,000 species (Hutchinson 1964). In India, this family is well represented with 132 genera and 925 species (Hooker 1879). The members of Leguminosae prefer diverse kinds of habitats in different regions of the world and vary in growth types from herbs to vines, shrubs and trees. Moreover, economically it is one of the most important families of flowering plants.

Reports are available on chromosome counts on a large number of genera (Senn 1938, Darlington and Janaki-Ammal 1955, Fedorov 1969, Sanjappa and Dasgupta 1983, Mukherjee and Sharma 1987). However, a perusal of literature shows that karyological studies are limited to mitotic analysis of root and in certain cases in shoot tip. No detailed karyological work has been done on embryo mitosis in this family.

The present investigation deals with eight different species of Legumiosae belonging to different genera in which analysis of somatic chromosome has been carried out both from developing embryo and root tip. It was desired to explore the extent to which the embryo so far considered difficult of study, could be utilized for chromosome analysis and to compare the manifestation of chromosome characteristics of embryo and root notwithstanding the basic similarity in the genetic make-up.

Materials and methods

The present investigation deals with the chromosome analysis of eight species of Leguminosae viz. *Lathyrus sativus* L., *Pisum sativum* L., *Crotalaria juncea* L., *Trigonella foenum-graecum* L., *Dolichos lablab* L., *Phaseolus mungo* Roxb., *P. vulgaris* L. and *Glycine max* Merr. Seeds were obtained from Oil and Pulse Research Institute, Berhampore as well as from local nurseries, Calcutta.

Well scattered metaphase plates were obtained by pretreating the root tips with para-dichlorobenzene–aesculine mixture (2: 1) for two to three hours at 10–12°C followed by overnight fixation in 1: 2 acetic ethanol. Usual acetic-orcein/N. HCl method was followed for staining.

In order to investigate the structure and behaviour of chromosomes of the embryo, pods of the right size were collected. The embryo at this stage was globular in shape. The ovule was halved longitudinally under a dissecting microscope and the embryo was carefully excised with the help of fine needle. The excised embryo was pretreated in para-dichlorobenzene–aesculine mixture (2: 1) for 2–3 hr at 12°C followed by fixation in Carnoy's fixative for overnight. Usual acetic–orcein/N. HCl method was followed for staining.

Observations

Cytological investigations of different species revealed a range of chromosome number

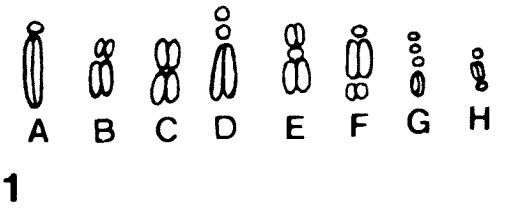

Fig. 1. Chromosome types

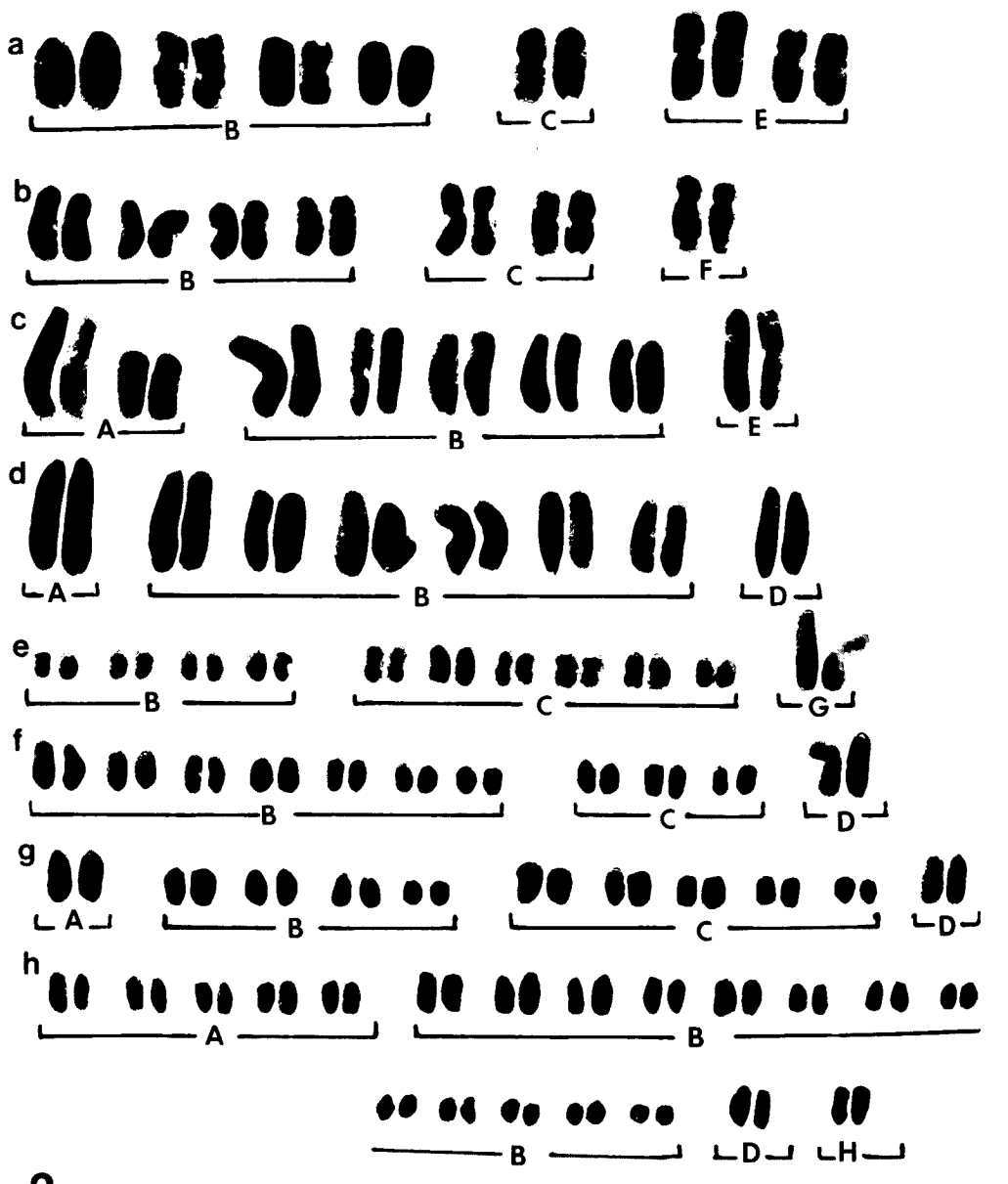

Fig. 2. Karyograms from root-tip cells (×2,000), (a) *L. sativus*, (b) *P. sativum*, (c) *C. juncea*, (d) *T. foenum-graecum*, (e) *D. lablab*, (f) *P. mungo*, (g) *P. vulgaris*, (h) *G. max*.

Table 1. Comparative chromosome characteristics of root-tip and embryo

Species	Chromosome number (2n)	Range of chromosome length (μm)		No. of chromosomes with secondary constriction		Chromosome types		Figure
		Root	Embryo	Root	Embryo	Root	Embryo	
Lathyrus sativus L.	14	5.5–3.5	6.5 –4.5	4	4	$B_8C_2E_4$	$A_4B_2C_4D_2E_2$	2a, 3a
Pisum sativum L.	14	5.5–4.5	7.5 –5.0	2	2	$B_8C_4F_2$	$A_2B_8C_4F_2$	2b, 3b
Crotalaria juncea L.	16	8.5–4.5	6.5 –5.0	2	2	$A_4B_{10}E_2$	$A_6B_4C_4E_2$	2c, 3c
Trigonella foenum-graecum L.	16	7.5–5.0	11.5 –4.5	2	2	$A_2B_{12}D_2$	$A_4B_{10}D_2$	2d, 3d
Dolichos lablab L.	22	4.5–1.0	3.5 –1.75	2	4	$B_8C_{12}G_2$	$A_2B_{16}D_2F_2$	2e, 3e
Phaseolus mungo Roxb.	22	4.0–1.5	2.75–1.5	2	2	$B_{14}C_6D_2$	$A_4B_{10}C_6D_2$	2f, 3f
P. vulgaris L.	22	3.0–1.5	2.5 –1.25	2	2	$A_2B_8C_{10}D_2$	$A_2B_{16}C_2D_2$	2g, 3g
Glycine max Merr.	40	2.5–1.0	2.5 –1.25	4	4	$A_{10}B_{26}D_2H_2$	$A_{14}B_{22}D_2H_2$	2h, 3h

from 14 to 40. The general types of chromosomes can be grouped into eight types (Fig. 1):
Type A: Extremely subterminal to nearly subterminal primary constriction.
Type B: Nearly submedian to submedian primary constriction.

Fig. 3. Karyagrams from embryo cells. (×2,000), (a) *L. sativus*, (b) *P. sativum*, (c) *C. juncea*, (d) *T. faenum-graecum*, (e) *D. lablab*, (f) *P. mungo*, (g) *P. vulgaris*, (h) *G. max*.

Type C: Nearly median to submedian primary constriction.
Type D: Two constrictions, primary and secondary. Of the three arms, one arm is longer than the other two which are more or less equal in size.
Type E: Tow constrictions, primary and secondary. Middle arm is shorter than the other

two arms which are unequal in size.

Type F: Two constrictions, primary and secondary. Middle arm is longer than the other two arms which are unequal in size.

Type G: Tandem satellite with three constrictions. One is extremely subterminal and the others are nearly submedian and nearly median.

Type H: Two constrictions, primary and secondary. Middle arm is longer than the other two arms which are equal in size.

Analysis of chromosome types of root tip cells and embryo cells of all the eight species revealed interesting data (Table 1). The number of chromosomes with secondary constrictions were noted to be almost similar in both the organs but minute differences in details of chromosome types recorded (Figs. 2, 3).

Discussion

Of the eight species studied, two are with 2n=14, two are with 2n=16, three are with 2n=22 and rest one with 2n=40 chromosomes. Different species with same chromosome number revealed differences in chromosome types including chromosomes with secondary constriction. This fact indiactes the role of structural alterations of chromosomes in this family.

It has been demonstrated that the tissues of the embryo in this family can conveniently be utilized for chromosome analysis. The method adopted has been found to be successful in all the species tried. The proper dissection of embryo however, is a prerequisite of the technique.

The manifestation of chromosome types of the embryo, however, is not necessarily identical with that of the root. Despite the fact that chromosome complements are essentially identical in morphology in all organs, their manifestation under the microscope may differ due to the penetrability of pretreatment agents and fixatives and the organs response to the processing steps involved. That is the reason why all the chromosome types in the embryo could not be matched in full with that of the root. Thus for a comparative study of the chromosome complements at interspecific and intergeneric level, the organ chosen for the study must remain identical.

Summary

Detailed analysis of chromosomes was undertaken in *Lathyrus sativus*, *Pisum sativum*, *Crotalaria juncea*, *Trigonella foenum-graecum*, *Dolichos lablab*, *Phaseolus mungo*, *P. vulgaris*, and *Glycine max* of Leguminosae with the aid of improved methods in both root and embryonic cell. Data on mitosis in tissues of embryo is hitherto unrecorded. The present investigation has demonstrated that with the technique devised, tissues of the embryo can be successfully utilized for chromosome analysis in this family. However, for the comparative study of the chromosome complements choice of similar organ is essential.

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