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INTRASPECIFIC NUCLEAR DNA VARIATION IN PIGEONPEA (*CAJANUS CAJAN* (L.) MILLSP.) AND PEA (*PISUM SATIVUM* L.)

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ABSTRACT

The nuclear DNA content was estimated in 13 varieties each of pigeonpea (*Cajanus cajan* (L.) Millsp.) and pea (*Pisum sativum* L.) using Fuelgen cytophotometry. Data were recorded on 25 2C and 4C nuclei in each accession with two replications. Significant differences were found among the varieties of the same species. DNA density (DNA content/nuclear area) increased with increase in DNA content. 4C nuclei exhibited higher density than 2C nuclei in each case. The 2C DNA values computed from 4C nuclei were slightly lower than those estimated from 2C nuclei directly.

Key words: Pigeonpea, pea, intraspecific variation, nuclear DNA content.

The nuclear DNA content has often been utilized for inferring evolutionary relationships [1, 2]. Such studies often assume constancy of DNA content within a species, which may not always be true. While massive variation in nuclear DNA content has been recognized at the interspecific level, the reports of intraspecific DNA variation have sometimes been questioned. For instance, intraspecific variation reported in *Picea glauca* [3] was questioned by Teoh and Rees [4] on the basis of a reinvestigation. While reviewing available data on nuclear DNA content in 753 species of flowering plants, Bennett and Smith [5] believed that upto 1% variation was observed within a species. However, there are a number of other reports of intraspecific variation in DNA which include those in the tribe Triticeae, e.g. *Aegilops squarrosa, Ae. longissima, Ae. sharonensis, Triticum monococcum,* and *T. timopheevi* [6–9]; *Pisum sativum* [10]; *Picea sitchensis* [11] and some *Allium* species [12]. More recently, intraspecific variation has also been reported in *Castus speciosus* [13], *Collinsia verna* [14], *Vetiveria zizanoides* [15], *Withania somnifera* [16], and several species of *Zea* [17].

Keeping this in view, possible intraspecific variation in DNA content is being examined by us in some leguminous crops. As a part of this study, we report in this communication the results of a study on the variation in nuclear DNA content among different genotypes of pigeonpea (*Cajanus cajan*) and pea (*Pisum sativum*).

MATERIALS AND METHODS

The seeds of thirteen genotypes each of pigeonpea (*Cajanus cajan*) and pea (*Pisum sativum*) were treated with 0.1% $HgCl_2$ for 4 min each and washed thoroughly in distilled water. They were germinated in an incubator at 30°C (*Cajanus cajan*) and 20°C (*Pisum sativum*) on filter paper moistened with distilled water. Primary young healthy root tips were excised and fixed in ice cold 4% neutral formaldehyde for 2 h [4], washed in distilled water for 24 h, with frequent changes and were fixed again in freshly prepared Carnoy's fixative (6:3:1) for 24 h. After washing in distilled water for 30 min, the root tips, hydrolysed in 5N HCl for 1 h at room temperature, were stained in Feulgen solution (pH 2.2) for 1 h. The stained root tips were thoroughly washed using three changes of SO_2 water for 10 min each, dried briefly on absorbent paper, and finally squashed firmly in a drop of glycerol.

Photometric measurement were recorded using Vicker's M85 microdensitometer within 3–5 h of squashing. For each material, about 25 well flattened 2C and 25 similar 4C nuclei were scanned in each of the two replications. All arbitrary relative absorption units were converted into absolute amounts using *Allium cepa* as standard (2C = 33.50 pg, [18]). The density of nuclear DNA was computed in arbitrary units by dividing the DNA value by the area of nucleus scanned.

RESULTS AND DISCUSSION

The nuclear DNA values of *Cajanus cajan* and *Pisum sativum* are given in Tables 1 and 2, respectively. The 2C nuclear DNA amount in pigeonpea ranged from 1.40 pg (P-852) to 2.18 pg (UPAS-120). Similarly, the range of 2C nuclear DNA in *Pisum sativum* was 7.83 pg (RP-78) to 9.84 pg (CUT-7P-888). In both species, thus, considerable variation exists for nuclear DNA content. The analysis of variance is presented in Table 3, which confirmed that the differences

Table 1. Nuclear DNA content and DNA density in *Cajanus cajan*

Genotype	Nuclear DNA (pg)		DNA density (DNA/area)	
	mean 2C value	mean 2C value computed from 4C	2C nuclei	4C nuclei
UPAS-120	2.18	2.15	0.131	0.134
H-2212	1.97	1.92	0.124	0.130
P-601	1.95	1.88	0.125	0.129
ICPL-8315	1.90	1.85	0.118	0.126
H-821	1.88	1.84	0.117	0.120
MUA-1	1.88	1.82	0.117	0.118
ICPL-151	1.84	1.80	0.114	0.118
AL-57	1.79	1.76	0.099	0.113
H-8122	1.74	1.73	0.092	0.109
H-8195	1.71	1.70	0.087	0.107
ML-15	1.61	1.56	0.083	0.101
MUA-2	1.51	1.46	0.081	0.100
P-852	1.40	1.37	0.075	0.095

among varieties were highly significant.

The variation in the nuclear DNA content may be associated with the divergence and evolution of species [19-23]. The factors leading to variation in nuclear DNA contents include addition or deletion of chromosome segments [24] and hybridization between species with large DNA differences [25, 26].

Table 2. Nuclear DNA content and DNA density in *Pisum sativum*

Genotype	Nuclear DNA (pg)		DNA density (DNA/area)	
	mean 2C value	mean 2C value computed from 4C	2C nuclei	4C nuclei
CUT-7P-888	9.84	9.76	0.290	0.388
PPMR-8	9.56	9.54	0.281	0.382
KPMR-39	9.04	8.99	0.260	0.379
Pant-P-8	8.98	8.95	0.253	0.350
KFP-104	8.72	8.70	0.252	0.342
KF P-4	8.68	8.63	0.250	0.332
KFP-103	8.63	8.56	0.250	0.330
KFPD-3	8.53	8.50	0.248	0.328
Rachana	8.51	8.45	0.241	0.325
H.U.P.-7	8.40	8.37	0.240	0.313
T-163	8.37	8.33	0.233	0.307
KPMR-22	8.05	8.01	0.219	0.290
R.P-78	7.83	7.82	0.193	0.272

In the present study, it is difficult to ascertain the mechanism of intervarietal DNA variation in *Cajanus cajan* and *Pisum sativum*. However, this study and other studies conducted in our laboratory on a variety of pulse crops, including chickpea [27] moong, urd and cowpea [28] provide definite evidence of intraspecific variation in nuclear DNA content. In another study on about 100 pea genotypes, 2-3 fold variation for nuclear DNA content was observed (S. N. Gupta, personal communication), and this variation in DNA contents is associated with large scale variation in karyotypes of these pea genotypes.

The nuclear DNA density in all the cases investigated was higher in 4C cells than in 2C cells (Tables 1, 2). This can be expected, if increase in DNA content is accompanied by greater DNA condensation. The 4C nuclei should have double the DNA content of 2C nuclei. In contrast to this expectation, in the present study, all the observed 2C values were slightly higher than those computed from 4C nuclei (See Tables 1, 2 [29]), indicating that the observed 2C values are overestimations relative to the 4C values.

Table 3. Analysis of variance of 2C nuclear DNA amount in *Cajanus cajan* and *Pisum sativum*

Source	d.f.	Mean squares	
		Cajanus cajan	*Pisum sativum*
Replications	1	0.00	0.003
Treatments	12	0.0775**	0.6540**
Error	12	0.01	0.0553
Total	25		

**Significant at P = 0.01.

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