

Table 2 Chi-square test for 1:1 and 1:0 ratios of normal vs puckerd leaves in the BC₁ and BC₂ generations of two reciprocal crosses of groundnut

Crosses	Leaf phenotype		χ^2
	Normal	Puckerd	
Backcross 1 (1:1 ratio)			
(J 11 × PLM) × PLM	21	13	1.88
(PLM × J 11) × PLM	14	7	2.32
(MK 374 × PLM) × PLM	12	3	4.27*
(PLM × MK 374) × PLM	11	9	0.2
Backcross 2 (1:0 ratio)			
(J 11 × PLM) × J 11	16	1	1.06 ^a
(PLM × J 11) × J 11	22	0	—
(MK 374 × PLM) × MK 374	10	0	—
(PLM × MK 374) × MK 374	25	1	1.04 ^a

*Significant at $P=0.05$.

^aThe χ^2 for testing 1:0 ratio was computed following $\chi^2 = m/n$, where n is the observed frequency in a normal class and m is the total.

(J 11 × PLM) × J 11 and (PLM × MK 374) × MK 374, in each of which one plant with puckerd leaves was also observed. However, the χ^2 value in both of these BC₂ crosses was nonsignificant at 0.01 probability. The occurrence of a single plant with puckerd leaves in BC₂ might be due to a chance mixing of seeds while processing the material.

The segregation pattern in F₂ of the two reciprocal crosses suggests that the normal leaf phenotype in groundnut is controlled by two pairs of genes, designated Nl_1 and Nl_2 . For the development of puckerd leaf character, the presence of the Nl_1 gene in recessive homozygous condition and the Nl_2 gene in dominant homozygous or heterozygous condition is essential. All other combinations will have normal leaves.

The backcross segregation pattern did not give strong support to F₂ observations when pooled analyses were done for a 1:1 ratio of normal vs puckerd leaves. Frequency of puckerd leaf plants was lower than expected in the backcross generation.

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A SIMPLE TECHNIQUE FOR POLLEN VIABILITY TEST

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A few techniques for testing pollen viability have been reported earlier¹⁻⁴. Of these, the method using sugar-based agar medium appears to be of wider use in the case of many plant species, including *Crotalaria juncea*⁵⁻⁷. Though growth of pollen tubes was different in different media⁵⁻⁷, there is also a report^{8,9} that pollen of *C. juncea* germinated well, in a very weak solution of sugar (cane sugar, 0.1 M). The present report describes a very simple test for pollen viability.

The test included four species, viz. *C. retusa* L., *C. juncea* L., *C. sericea* Retz and *C. verrucosa* L. After anthesis the pollen was collected and dusted on clean, grease-free slides. Just after dusting, one or two drops of distilled water were added to the pollen mass and the pollen was spread carefully with the blunt end of a dissecting needle. After proper labelling each processed slide was placed on match sticks in petri dishes containing a small piece of moist blotting paper. The petri dishes were kept covered to maintain humidity. A parallel set of slides with pollen in cane sugar solution (0.1 M) was also set up. Pollen from both water and cane sugar medium was examined microscopically every hour. The material was stained with a drop of glycerine-acetocarmine mixture (1:1) and covered with a cover glass before observation. Pollen germination was recorded from ten samples and tube growth from 20 pollen grains picked at random.

Pollen germination in distilled water was almost as good as that in sugar solution (0.1 M) in the case of *C. retusa*, *C. juncea* and *C. sericea*, while the *C. verrucosa* there was a significant difference (table 1). *C. juncea* pollen tube growth in distilled water was significantly less than that in sugar solution (table 2). The above observations show that while distilled water alone cannot substitute for sugar solution or other sugar-based medium with regard to pollen

Table 1 Germination of *Crotalaria* pollen in cane sugar solution and distilled water under room temperature conditions

Species	Sugar solution (0.1 M)		Distilled water		t
	Range (%)	Mean (%)	Range (%)	Mean (%)	
<i>C. sericea</i>	82.14–100	92.00 ± 1.80	75.60–94.59	86.00 ± 2.05	2.19*
<i>C. retusa</i>	92.30–100	96.03 ± 0.64	92.23–98.00	94.37 ± 0.55	1.95*
<i>C. juncea</i>	90.90–100	96.49 ± 1.06	89.65–100	94.91 ± 0.94	1.40 ^{NS}
<i>C. verrucosa</i>	91.89–100	94.45 ± 0.85	62.74–86.66	75.03 ± 2.28	7.96**

*Significant at 5%; **Highly significant; NS, not significant.

Table 2 *Crotalaria juncea* pollen tube growth in sugar solution and distilled water

Incubation time (h)	Sugar solution (0.1 M)		Distilled water		t
	Range (µm)	Mean (µm)	Range (µm)	Mean (µm)	
1	6–12	9.5 ± 0.38	4–10	7.5 ± 0.40	3.59
2	20–38	27.00 ± 0.96	6–20	12.5 ± 0.80	11.51
3	26–50	41.1 ± 1.61	12–22	17.1 ± 0.62	13.84
5	40–68	58.1 ± 2.12	22–48	31.00 ± 1.82	9.63
24	62–84	70.4 ± 1.58	24–50	32.4 ± 1.63	16.65

tube growth and development, it is sufficient for germination of pollen grains in some species, for which this method may be attempted.

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A NOTE ON THE ANEUPLOIDY IN *ARAIOSTEGIA PULCHRA* (D. DON) COPEL. AND *ASPLENIUM YOSHINAGAE* MAKINO VAR. *PLANICAULE* MORTON

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ASPLENIUM YOSHINAGAE var. *planicaule* (Aspleniaceae) and *Araiostegia pulchra* (Davalliaceae)¹ were collected from Damthong at 2200 m in South Sikkim and Aritar at 1200 m in East Sikkim. *A. pulchra* grows on tree trunks and *A. yoshinagae* var. *planicaule* at the foot of tree trunks under moist and shady conditions. Occasionally *A. pulchra* is found on damp rocks under trees on accumulated humus. Both ferns prefer epiphytic habitat.

Fixation of young sporangia was done in 1:3 acetic acid:alcohol on the spot. Acetocarmine (2%) was used as the staining reagent in squash preparations. At least 15 cells were analysed to obtain the chromosome number.

A. pulchra showed 41 bivalents at meiosis (figures 1 and 2). The earlier reports on it, however, are²⁻⁴ $n=40$. *A. yoshinagae* var. *planicaule* exhibited 40 bivalents in spore mother cells (figures 3 and 4). This chromosome number, reported for the first time in the species, is comparable only to *A. unilaterale* Lam., where Bir⁵ reported $n=40$ in plants from East Himalayas. *A. yoshinagae* var. *planicaule* differs