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Short Communication

Inheritance of functional male sterility in the medicinal plant, periwinkle

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Periwinkle [*Catharanthus roseus* (L) G. Don], an ever blooming tropical plant, is the source of anti-cancer (vincristine and vinblastine) and anti-hypertension (ajmalicine) alkaloids, which are found in its leaves and roots, respectively. Levy *et al.* [1] reported heterosis for leaf and root yields while Sevestre-Rigouzzo *et al.* [2] reported heterosis for the content of alkaloids in leaves and roots. Automatic intra-flower self-pollination does not occur in periwinkle and pollination is brought about mainly by butterflies. Periwinkle produces fairly large number of flowers per plant per day, and pollinating butterflies have been found to bring about geitonogamous self-pollination as well as phenotypic assortative mating for flower colour [3]. Therefore, although periwinkle is a crosspollinating species, the availability of male sterility is an essential pre-requisite for exploiting heterosis. A mutant (EMS 1-10) with indehiscent anthers (functional male sterility) was isolated following an induced mutagenesis programme, which was taken up with an objective of inducing mutants with high content of alkaloids in a dieback resistant variety, Nirmal [4]. The mutant appeared to be similar to another mutant, wavy leaf margin (NEU 1-7), except for indehiscent anthers. The mutant was phenotypically normal for other morphological traits. The mutant had smaller anthers (Fig. 1) with lesser number of pollen grains than parental variety, Nirmal. Pollen grains of the mutant were fertile, and good fruit- and seed-set was obtained on artificial selfing (Table 1).

The mode of inheritance of indehiscent anthers was studied by crossing the mutant with the parental variety, Nirmal. Crosses were made in the glass house as described earlier [4]. F_1 plants were selfed artificially to produce seeds for raising the F_2 generation and were also back crossed to parents to produce seeds for raising the back cross generations. Segregation data of F_2 and backcross generations suggested that the indehiscent anthers trait was controlled by a single recessive gene, which was found to be linked to the gene controlling wavy leaf margin (Table 2). The estimates of recombination fraction (p) between the two genes calculated by maximum likelihood method [5] from F_2 and testcross data were 29.0 ± 4.28 and 29.6 ± 6.21, respectively. Gene symbols *ia* and *wy*, are proposed for the genes governing indehiscent anthers trait and wavy leaf margin, respectively. This is the first report on linkage in periwinkle.

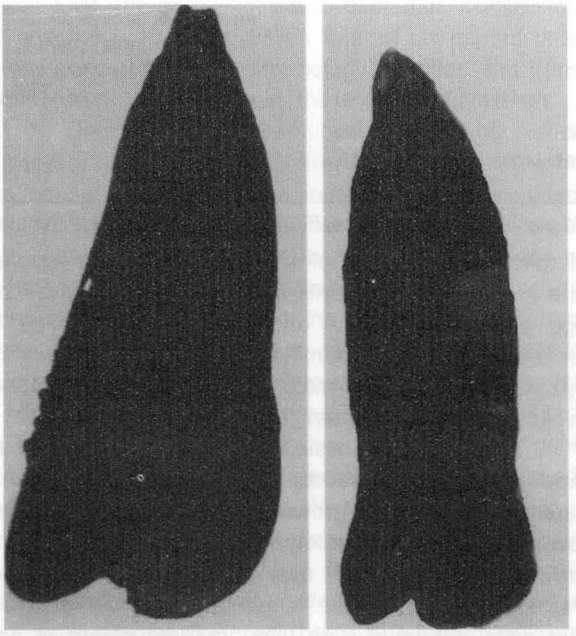

Fig. 1. Anthers of the mutant EMS 1-10 (right) and its parental variety, Nirmal (left)

Functional male sterility i.e., where dehiscence of anthers does not occur due to the prevention of opening

Table 1. Some characteristics of the mutant, EMS 1-10 with indehiscent anthers and its parental variety, Nirmal of periwinkle

Character	Nirmal	EMS 1-10
	Mean ± SE	Mean ± SE
Pollen fertility (%)	98 ± 0.68	96 ± 4.1
Pollen germination (%)	95 ± 0.61	94 ± 0.60
Anther length (mm)	2.91 ± 0.01	2.71 ± 0.01
Anther breadth (mm)	1.14 ± 0.01	0.87 ± 0.01
Number of pollen grains/anther	1733 ± 49.5	1179 ± 35.7
Per cent fruit set on artificial selfing	94.0	90.0
Follicle length on artificial selfing (cm)	3.1 ± 0.06	2.9 ± 0.06
Seeds/follicle on artificial selfing	27 ± 0.7	16 ± 1.2
100 seed weight (mg)	131 ± 0.5	115 ± 0.7

SE = Standard error

Table 2. Segregation of indehiscent anthers and wavy leaf margin in the F_2 and backcross generations of the cross involving mutant EMS 1-10 (with wavy leaf margin and indehiscent anthers) and parental variety, Nirmal of periwinkle

Generation	Phenotypic class		Total	Expected ratio	$\sum\chi^2$	P
	Normal anthers	Indehiscent anthers				
F_2	121	48	169	3:l	1.024	0.50-0.30
Backcross* (F_1 × EMS 1-10)	25	29	54	1:1	0.296	0.70-0.50
	Normal leaves	Wavy leaf margin				
F_2	120	49	169	3:1	1.415	0.30-0.20
Backcross (F_1 × EMS 1-10)	29	25	54	1:1	0.296	0.70-0.50

Joint segregation of indehiscent anthers with wavy leaf margin

	Normal leaves and normal anthers	Normal leaves and indehiscent anthers	Wavy leaf margin and normal anthers	Wavy leaf margin and indehiscent anthers				
F_2	98	22	23	26	169	9:3:3:1	28.325	< 0.001
Backcross (F_1 × EMS 1-10)	19	10	6	19	54	1:1:1:1	9.553	0.05-0.02

*All plants of the back cross, F_1 × Nirmal, had normal anthers and normal leaves

of thecae or absence of stomium, has been reported in a number of crop plants such as, sweet pea, tomato, egg plant, barley etc., and has been controlled by recessive as well as dominant genes [6, 7]. Recently, cytoplasmic functional male sterility has been reported in *Allium schoenosrasum* [8].

To study the possibility of using this mutant for production of hybrid seeds, the mutant and the parental variety, Nirmal were grown in a plot of 10 m^2. One row of the parental variety, Nirmal (male parent) was grown on either side of every two rows of the mutant. Percent fruit set, follicle length and number of seeds fruit were determined on thirty random plants of both male (variety, Nirmal) and female (male sterile mutant EMS 1-10) rows. High fruit-and seed-set was observed on plants of the male sterile mutant (Table 3). All seedlings (1167 in number) raised from a sample of the seeds collected from plants of the mutant EMS 1-10 were found to be hybrids. Since the mutant EMS 1-10 had wavy leaves, a monogenic recessive trait [4], the hybrid nature of seedling could be easily inferred from their normal leaves. The mutant EMS 1-10, in addition to wavy leaves, has three more recessive traits *viz.*, green hypocotyl, pubescent leaves and white flowers. The traits wavy leaves, purple hypocotyl (the contrasting dominant trait of green stem) and pubescent leaves are expressed as early as in 30 day old seedlings. The use of dominant alleles at one or more of these three loci in the male parent should facilitate easy identification of true hybrids at the seedling stage in the nursery. Since periwinkle is generally grown as a transplanted crop, only true hybrid seedlings can be transplanted in the field. This mutant can be easily multiplied by artificial selfing, vegetatively through stem cuttings or micro-propagation. Its practical utility in the production of hybrid seeds would obviously be determined by the economics of its multiplication and heterotic advantage of the hybrid.

Table 3. Values of mean ± standard error of per cent fruit set, follicle length and number of seeds per follicle in the male sterile mutant EMS 1-10 (female parent) and parental variety, Nirmal (male parent) on open-pollination in the field

	Fruit set (%)	Follicle length (cm)	Number of seeds/follicle
EMS 1-10	81.9 ± 2.7	2.2 ± 0.1	15.1 ± 1.3
Nirmal	85.9 ± 3.6	2.9 ± 0.1	24.5 ± 0.9

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