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Genetics and mechanism of induced male sterility in *Andrographis paniculata* (Burm. f.) Nees and its significance

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***Andrographis paniculata* is a multipurpose plant of immense therapeutic value and wide geographic distribution. Here we demonstrate the induction of genic male sterility with γ-irradiation. Male sterility was conditioned by a single recessive genic mutation that acted upon the tapetal layer surrounding the pollen sac and was also manifested as hypertrophied non-sporogenous tissue, invading the anther locule. Female fertility remained unimpaired and fully intact. We also elucidate the consequences and significance of intervarietal hybridization employing male sterile line as one of the parents. Positive heterosis in many yield attributes and metabolic pathway elaboration/intensification exhibited is suggestive of high breeding value of the genic male sterility developed in *A. paniculata*.**

Keywords: *Andrographis paniculata*, andrographolide, genic male sterility, medicinal plant, obligate selfing.

ANDROGRAPHIS paniculata (Acanthaceae) reputed as 'Kalmegh' or 'green chiretta' is a medicinal herb of immense therapeutic value in Ayurvedic, Unani and Siddha medicines, the traditional medicinal systems of India[1,2]. It is an annual herb (Figure 1 *a*), native to peninsular India and Sri Lanka, and naturalized throughout the warmer and humid parts of India[1]. The species is also reported from different phytogeographical and edaphic zones of Southeast Asia, China, America, West Indies and Christmas Island in Indian Ocean. 'Kalmegh' which consists of dried leaves and tender shoots is used as a remedy for various ailments related to digestion, hepatoprotection, antipyretic, vermicidal, antiacne, analgesic, anti-inflammatory, antibacterial, antityphoid, antibiotic and hypoglycaemic activities, besides immunity enhancement[3–7]. There are about a dozen polyherbal formulations of this plant being used as a hepatoprotective. Its therapeutic value is ascribed to various bioactive molecules synthesized and accumulated in its leaves and roots. Some of the major bioactive constituents include andrographolide and related diterpenes, i.e. deoxyandrographolide, 14-deoxy-11,12-didehydroandrogra-

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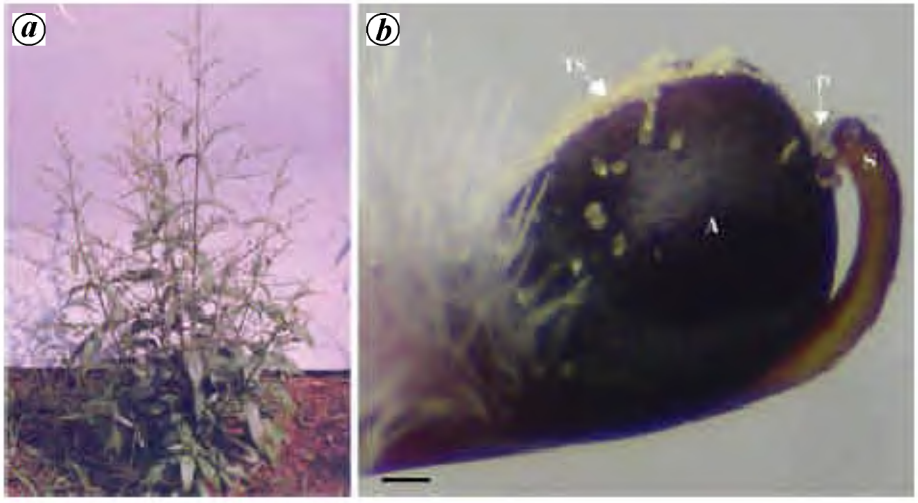

Figure 1. Plant habit: Indeterminate shoot of *Andrographis paniculata* (***a***) Pollen (P) clogged stigma (S) adpressed to transversely (TS) dehiscent anthers (A) provides for obligate autonomous selfing (***b***) (bar = 120 μm).

pholide, neoandrographolide and andrographiside. In recent years there has been a noticeable surge in its pharmacological studies. It has been shown that andrographolide protects liver and gall bladder and is more efficacious than silymarin, a known hepatoprotective drug[8]. Neoandrographolide has shown greater activity against malaria[9] and is hepatoprotective against carbon tetrachloride-induced hepatic damage[10], while 14-deoxyandrographolide produces a more potent hypotensive effect in anesthetized rats and in isolated right atria[11]. The crude extract from whole plant has also revealed anti-HIV activity[12].

A. paniculata is hermaphroditic, self-compatible and a habitual inbreeder. Intimate proximity of adpressed stigma with the anthers (Figure 1 *b*) and synchronization of anther dehiscence and stigma receptivity, provide for obligate autonomous selfing in the species. It exhibits a number of features that are in conformity with its breeding behaviour (summarized in Table 1).

Classical means of propagation of *A. paniculata* is through seeds. Under cultivation, seed-to-seedling and seedling-to-seed durations are 30 and 140 days respectively. It displays considerable variability in its morphometric and chemical traits[13]. As the species is predisposed for selfing due to its floral architecture and overlap of male and female phases, the present study was aimed to explore the possibility of inducing male sterility in order to emasculate the flowers genetically with a twin objective of optimizing its genetic amelioration, and also to enrich our germplasm resource base.

We tested individual open-pollinated seed-lots of 12 accessions for their vulnerability to different chemical and physical mutagens, singly and in combination for the induction of male sterility. Pre-soaked seeds in deionized water overnight at 25 ± 2°C of one of the accessions, APJ 013, on exposure to 20 kR γ-irradiation, yielded male sterile mutants in M_1 progeny. The data obtained (not given) over a period of three years (2001–04) suggest that the locus within APJ 013 is highly susceptible to mutation as the frequency of male sterile mutants obtained ranged from 6 to 14%. Preliminary investigations also indicate that out of 53 accessions available in our germplasm repository, so far APJ 013 seems to enjoy the monopoly to mutate and produce male sterile plants.

Hand-pollination of male sterile plants with genotypically different male fertile accessions revealed that a single recessive mutant gene conditions this anomaly. This was illustrated by segregation of F_2 progenies, conforming to the Mendelian segregation frequencies of observed and expected frequencies (3 : 1) in the usual method by means of χ^2 goodness-of-fit test ($\chi^2 = 1.558$, $P = 0.25–0.20$; Table 2). In the male sterile progenies, we could not establish any pleiotropic and/or linkage phenotypic markers associated with genic sterility. The only reliable criterion to identify male sterile plants was by unstained, deformed and shrunken pollen.

Male sterility in *A. paniculata* is of sporogenous-type in which the release of microspores from quartets and tapetal degeneration is simultaneous (Figure 2 *a*, *b*). Furthermore, non-sporogenous tissue is also acted upon by genic mutation. This is manifested by the hypertrophied sterile tissue (Figure 2 *b*) within the anther locule. This anomaly results in encroachment of the locule, thus significantly reducing the pollen production in male sterile flowers (Table 1). Microspore abortion is characterized by mild exine differentiation, abortive and shrunken pollen (Figure 2 *c*, *d*) and cytoplasmic and nuclear degeneration. Somatic chromosome count is 50. Until the release of microspores, meiosis precedes normally with perfect metaphase bivalent congression, orientation and normal 25 : 25 disjunction at anaphase I and II. Precocious tapetal breakdown seems to be the only cause for male sterility in the present study. It is amply established that growth and maturation of micro-

Table 1. Comparative summary of morphometric traits and reproductive parameters in parents and hybrid of *Andrographis paniculata*

Character	Male fertile (APJ 013)	Male sterile (APJ 013)	Male sterile × male fertile (APJ 013) (APJ 020)
		M_1 progeny	F_1 progeny
Total shoot biomass/plant (g) [*B*]	452.64 ± 8.80* (25)**	468.08 ± 5.34 (25)	641 ± 6.40 (25)
Leaf count/plant	327.38 ± 5.84 (25)	303.90 ± 4.21 (15)	443.56 ± 5.03 (15)
Leaf/stem ratio/plant	0.387 ± 0.006 (25)	0.401 ± 0.002 (25)	0.372 ± 0.005 (15)
Leaf area/plant (cm^2)	1613.98 ± 36.40 (15)	1674 ± 24.37 (10)	2325.38 ± 21.2 (10)
Dry leaf biomass/plant (g) [*D*]	44.11 ± 2.97 (25)	45.36 ± 2.30 (10)	61.28 ± 1.66 (10)
Plant height (cm)	60.56 ± 3.87 (25)	56.23 ± 1.32 (10)	43.78 ± 2.04 (10)
Plant spread (cm)	48.10 ± 2.62 (25)	49.38 ± 1.48 (10)	54.21 ± 1.97 (10)
Floral biomass/plant (g) [*F*]	6.90 ± 0.012 (12)	7.04 ± 0.007 (10)	9.74 ± 0.007 (10)
Reproductive effort [*RE*] [*B* + *F*]/[*D*]	10.42	10.47	10.62
Seed weight/cm^2 leaf area (mg)	7.4	0.019	9.4
Total seed weight/plant (g)	11.963	0.032	21.02
1000 seed weight (g)	1.612 ± 0.032 (10)	1.863 ± 0.40 (5)	2.052 ± 0.04 (10)
Seed set percentage			
Open-pollination (natural)	85.36 ± 0.64 (300)	1.96 (400)	0
Spontaneous self-pollination (bagging)	84.42 ± 1.02 (215)	0	0
Xenogamy (manual cross-pollination)	Not performed†	82.08 ± 2.11 (470)	82.08 ± 2.11 (470)
Seed germinability (%)	86.23 ± 4.67 (1000)	89.36 ± 4.21 (450)	91.13 ± 6.37 (1000)
Number of pollen grains/flower	4215.60 ± 126.70 (10)	1738 ± 237.60 (15)	4429.31 ± 168.00 (10)
Pollen stainability (%)	78.30 ± 9.23 (2000)	1.96 ± 0.03 (2000)	82.72 ± 3.12 (2000)
In vitro pollen germinability (%)	61.37 ± 6.14 (570)	0	63.78 ± 3.09 (830)
Pollen size (μm)	84.60 ± 2.24 (350)	Shrivelled	83.07 ± 2.83 (500)
Number of ovules/flower	12 (20)	12 (20)	12 (20)
Pollen/ovule ratio [*P*/*O*]	351.50	144.83	369.11
Number of flowers/plant [*Fl*]	762 ± 23.87 (10)	769 ± 21.04 (10)	1045 ± 42.17 (10)
Number of fruits/plant [*Fr*]	724.00 ± 21.70 (10) (OP)***	123.00 ± 11.30 (10) (OP)	996.41 ± 17.00 (10)
Number of seeds/fruit [*S*]	10.25 ± 1.48 (350) (OP)	0.163 ± 0.002 (400) (OP)	10.28 ± 0.90 (415)
Seed/ovule ratio [*S*/*O*]	0.85	0.014	0.86
Fruit/flower ratio [*Fr*/*Fl*]	0.95	0.160	0.95
Preemergent reproductive success [*Fr*/*Fl* × *S*/*O*]	0.81	0.002	0.82

*Mean and SEM; **Number of observations; ***Open-pollination.

†Due to intimate proximity of minute reproductive parts (Figure 1 *b*) and their vulnerability to mechanical injuries during manual emasculation.

Table 2. Inheritance pattern of male sterility in *A. paniculata*

Generation	Observed number		Expected number		Ratio tested	χ^2	*P*
	Male fertile	Male sterile	Male fertile	Male sterile			
F_1	46	0	46	0	1 : 0	–	–
F_2	158	56	160.5	53.5	3 : 1	1.558	0.25–0.20

Table 3. Comparative chemical profiles* of parents and hybrid

Bioactive constituent	Male sterile (APJ 013)	Male fertile (APJ 020)	Male sterile × male fertile (APJ 013) (APJ 020) F_1 progeny
Andrographiside	0.21 ± 0.02	0.56 ± 0.02	0.96 ± 0.03
Andrographolide	0.92 ± 0.06	1.78 ± 0.05	3.70 ± 0.07
Neoandrographolide	0.88 ± 0.06	1.10 ± 0.04	1.58 ± 0.04
14-deoxy-11,12-didehydroandrographolide	0.57 ± 0.03	0.71 ± 0.02	1.09 ± 0.02
Total andrographolides†	2.58	4.14	7.33

*Andrographolides were determined according to HPLC method of Saxena *et al.*[7] after 120 days of transplantation.
† Data represent mean of five plants from each parent and hybrid (F_1) progeny.

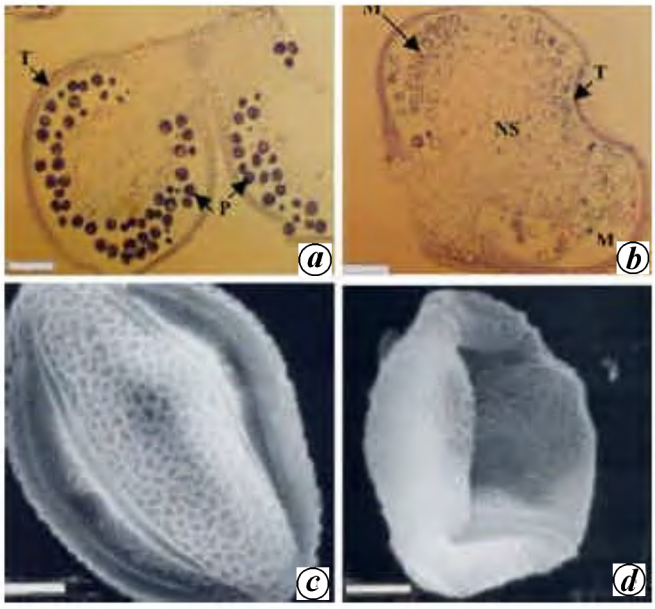

Figure 2. Semithin sections of bisporangiate fertile and sterile anthers: Stained viable pollen (P) and intact tapetum (T) of male fertile anther (bar = 100 µm) (***a***), unstained aborted microspores (M), hypertrophied non-sporogenous tissue (NS) invading the locule of male sterile anther (bar = 100 µm) (***b***), scanning electron micrographs of fertile pollen (bar = 5 µm) (***c***) and of sterile pollen (bar = 5 µm) (***d***).

spores is solely dependent on the normal behaviour of the tapetum. It ensues the supply of nutrients, hormones, enzymes and also the exine contents through ubisch bodies to the heterotrophic microspores[14,15]. Thus premature degeneration of tapetum is always coupled with pollen abortion.

A comparative summary of various morphometric variables and reproductive parameters obtained from male fertile, male sterile and manually pollinated male sterile × male fertile (F_1) is shown in Table 1. Perusal of data revealed that there were no significant differences between male fertile and male sterile progenies in their morphometric attributes. However, reproductive parameters such as pollen production and consequently pollen–ovule ratio were significantly reduced. There was no seed set on spontaneous selfing (bagging), confirming complete male sterility. Female fertility remained unimpaired and fully intact, as elucidated by xenogamous pollination (Table 1). Reduction in pollen production was not accompanied by any significant increase in resource allocation to female reproductive components. This is possibly due to the considerable time lapse between the moment of saving and of investment in maternal structures as the male sterile mutations act late on the anther development, so that the savings on paternal costs are likely to be small[15–17].

Manual cross-mating facilitated as a consequence of genetic emasculation of APJ 013 with male fertile APJ 020 as a pollen donor, yielded F_1 progeny that exhibited positive heterosis in many yield attributes over both the parents (Table 1). About 39% increase in dry leaf biomass, 22% higher seed output per unit leaf area, improved seed germinability (91.13%), etc. are testimony to the immense breeding value of induced genic male sterility in *A. paniculata*. The progeny obtained was also quantitatively different from both parents with regard to bioactive constituents (Table 3). There was appreciable enhancement in the concentration of all the constituents and overall increase of more than two-fold in the yield of total andrographolides (7.33%) when compared with the mean value (3.215%) of both parents. The secondary pathway elaboration/intensification may be either due to gene complementarity or additive

effects or even dominance. Inheritance pattern of secondary metabolites is always complex due to the involvement of many allelic and non-allelic genes. Nevertheless, the point worth making about the present results is that, the genic male sterility offers tremendous scope for qualitative and quantitative improvement of *A. paniculata* through intervarietal hybridization.

Examples abound where the heterozygote advantage of F_1 progeny due to single recessive genic male sterility has been exploited commercially for improvement in the productivity of a variety of crops[15,18], i.e. cereals, legumes, oil seed crops, etc. Contrary to this, there seems to be too little studies and scarce published data regarding the applied and basic aspects of male sterility in medicinal plants. The male sterile lines at present constitute an important genetic resource in our germplasm repository. It throws a fresh perspective for metabolic engineering by changing the combinations of genotypic lines with different range of bioactive andrographolide constituents to fulfil the shifting demands of market. We now tend to assess the combining ability of diverse genotypes with the sterile lines. Literature survey shows that ours is possibly the first demonstration of induced genic male sterility and its significance in genus *Andrographis* and probably also the first in family Acanthaceae.

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Discovery of nannofossils in a plant bed of the Bhuj Member, Kutch and its significance

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The world-famous Mesozoic rocks of Kutch exposed in the western sector of India have been originally classified into the Patcham, Chari, Katrol and Umia formations in ascending order along with a later scheme of Jhurio, Jhumara, Jhuran and Bhuj formations. Ammonites are the best for dating marine Mesozoic succession the world over. The Kutch Mesozoic succession has been well dated through ammonoids but for its youngest ammonoid-devoid Bhuj Member, of Umia Formation, which is otherwise essentially made up of thick, bioturbated, coarse-grained sandstone and grits with enrichment of iron at places and leaf-bearing carbonaceous shales at partings. No other marine body fossils are known in the Bhuj Member but for disputed foraminifers. The Bhuj Member had been for long considered a continental or freshwater deposit by several workers. This view was contested by later workers on the basis of the presence of profuse and thick ferruginous bioturbated horizons, facies analysis, etc. However, in the absence of datable marine

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