Table 1. Water quality parameters of ADS-affected ponds

Parameter	Range	Average (mean ± SE)
pH	7.4–8.4	7.79 ± 0.11
Total alkalinity (mg/l, as $CaCO_3$)	50–400	270 ± 35.68
Total hardness (mg/l, as $CaCO_3$)	40–245	155.9 ± 19.29
NH_4-N (mg/l)	0.01–1.70	0.64 ± 0.22
NO_3-N (mg/l)	0.005–0.60	0.24 ± 0.08
Dissolved phosphate (mg/l)	0.02–0.08	0.05 ± 0.01
Conductivity (mmho/cm)	0.73–2.47	1.61 ± 0.31

Data are mean of 12 samples collected from different ponds/borewells of ADS-affected regions of Nellore.

larly, the histology of muscle tissue or hepatopancreas did not reveal any significant pathology.

On the other hand, major alterations in the physico-chemical parameters of the water samples were observed, which may not be suitable for scampi culture on long-term basis (Table 1). The observed high alkalinity, hardness and ammonia content in the cultured water, where the culture is mostly practised on a continuous basis without intermittent drying of the pond, might have occurred due to high organic load in the pond. Most of these parameters observed in the culture ponds are beyond the recommended levels of freshwater prawn farming. Ideal levels of transparency (25–40 cm); alkalinity ($CaCO_3$; 20–60 mg/l); hardness ($CaCO_3$; 30–150 mg/l), and ammonia (0.1–0.3 mg/l) in *M. rosenbergii* culture ponds are essential[7]. The role of water quality parameters in the sampled ponds with relation to the occurrence of ADS needs to be further investigated.

Similar type of culture technology is also practised in other parts of Andhra Pradesh, particularly Kakinada and Vijayawada areas, using the same seed sources and feed materials. However, occurrence of ADS has not been reported so far. The low rainfall in Nellore region, forcing farmers to exploit underground source of water and continous culture in the same pond, might be playing roles as predisposing factors or causal factors for ADS. However, further in-depth study is required to confirm involvement of any pathogen or carotenoid deficiency or water quality as the possible cause(s) of ADS.

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Reproductive biology of *Withania somnifera* (L.) Dunal

Withania somnifera (family Solanaceae) is an important medicinal plant known classically for its rejuvenating properties, and hence called Indian Ginseng[1]. In view of its varied therapeutic potential, it is the subject of considerable modern scientific attention. A perusal of the literature shows that little information exists on floral biology and reproductive behaviour, and there is emerging need to select high-yielding plants and genetically improve them for cultivation to meet the demand of the pharmaceutical industries. Some crosses between two cultivated lines (WS20 and WS22) with wild type were attempted for increase in root yield and seed yield over their parents[2–4].

In our institutional experimental farm, we have a wide genetic resource base of the species constituting 50 accessions collected from all over India. Morphological and chemical characterization of these accessions resulted in identification of some elite types, which will eventually help in evolving varieties of commercial significance. The present investigation was undertaken to study the morphological and functional characteristics of the flowers contributing towards the reproductive success of the best performing morphotype – AGB002 (a selectant originated from wild populations in Rajasthan region) growing in experimental plots of our institute.

Pollen transfer experiments were carried out to investigate the effect of pollen source on fruit set and number of seeds/fruit (seed set) as well as seed germination. The treatments tried were: (i) autogamy – pollination with pollen from the same flower ($n = 20$ flowers on 20 plants); (ii) open pollination – 20 plants were tagged without receiving any treatment and (iii) xenogamy – pollination with pollen from another accession AGB025 (cultivated variety) followed by bagging ($n = 20$). Pollination was manually undertaken during 0900 to 1100 h (period of high stigma receptivity) during April to May 2003. In all the cases, once the necessary time had elapsed, fruit set and seed set

were recorded. Seeds from different treatments were tried for germination one month after collection (moisture level 7–9%).

Pollen count was estimated on anthers collected just prior to anthesis from mature flowers, kept in a small vial in 1% glycerine, finely chopped, spun on vortex mixer and pollen grains counted with a haemocytometer[5]. Pollen viability estimates were made by TTC (2,3,5-triphenyl tetrazolium chloride) test[6]. Plumpy, deeply stained pollen grains were treated as viable, while small, empty, shrunken and poorly stained as non-viable. Ovule count was made from the ovary collected at anthesis and the number of ovules counted after dissection under a stereomicroscope (20x).

The morphotype AGB002 is a perennial shrub, 120–130 cm tall and having sharply acute, slightly hairy membranous leaves with entire margin. Shoots 6–7 arising from a basal crown and 2 floral shoots at each node. Flowers 7–15 in axillary clusters. The flowers are pentamerous, antinomorphic, hypogynous and hermaphroditic. Each flower bears 5 epipetalous stamens and a pistil. The ovary is bilocular with axile placentation. The berry colour is always red. The species exhibits perpetual flowering (throughout the year) with a peak period in April–June (Table 1). Another contrasting accession–AGB025 is an annual, rarely more than 45 cm tall and having small, densely pubescent, subcoriaceous leaves with undulate margins. Many small shoots arise from the basal crown. The berry colour is invariably orange.

Table 1. Floral morphology/effort for male–female function in *Withania somnifera*

Character	Observation
Flowering	Peak April–June (flowers appearing throughout the year)
Flower type	Pentamerous, hermaphrodite
Colour	Dull green
Odour	Negligible
Anthesis	0900 to 1100 h
Anther dehiscence time	0900 to 1100 h
Anther dehiscence mode	Longitudinal
Stigma receptivity	Synchronous with anther dehiscence
No. of anthers/flower	5
No. of pollen grains/anther	5800 ± 175.7
No. of pollen grains/flower (estimated)	29,000
Pollen viability (%)	65–70
Pollen shape	Spherical to sub-spherical
Stigma type	Papillose
No. ovules/flower	35.5 ± 5.85
Pollen: ovule ratio/flower	817 : 1
Berry colour	Red (in accession under study)

Figure 1. *a*, Habit; *b*, Inflated calyx at fruiting; *c*, Fruit exposed; *d*, Flower at anthesis (stigma protruding); *e*, L.S. of flower at anthesis; *f*, Flower at anther dehiscence (stigma enclosed); *g*, L.S. of flower at dehiscence.

The expansion of petal lobes at the tip heralds anthesis, which occurs at 0900 to 1100 h. During anthesis, the carpel is longer than the stamens, but gradually the stamens elongate after 3–4 days as anther dehiscence sets in, reaching equal or slightly greater height than the carpel. A signal of stigma receptivity is marked visually by elongation of the filament in the stamens of 95% flowers observed. However, in 5% flowers the stamen length was the same as that of the carpel at anthesis. The filaments are inserted into stapets or stirrups, which are fused with the corolla tube. The stapet (filament base) is appressed to the ovary and a groove between each stapet allows nectar to flow upwards from the nectary at the ovary base. Anther dehiscence and stigma receptivity synchronize creating conditions conducive to selfing/autogamy. When receptive, the stigma surface appears fresh, shining, papillose, greenish and slightly sticky. Anthers dehisce longitudinally on the inner side only. They more or less cover the stigma to ensure sufficient pollen deposition on it (Figure 1). Synchronous stigma receptivity, anther dehiscence, relative length and close proximity of anthers and stigma predispose this species for autogamy.

The manual-pollination experiments revealed predominant self-compatible nature of the species (Table 2). Autogamy and open pollination resulted in high fruit/seed set (81.83% ± 1.94 and 86.66% ± 1.74; 83.10% ± 3.90 and 87.30% ± 3.08 respectively). Xenogamy (controlled crossing) led to very low fruit set: average 3.59% ± 0.85 (for 002 × 025), 6.50% ± 0.84 (for 025 × 002) and 10% ± 1.06 ((for 002 × 002). The seed set r ecorded was 30.22% ± 5.85 (for 002 × 025),

Table 2. Fruit/seed set and germination in different pollination treatments

Pollination treatment	Accession code	Fruit set (%)	Seed set/berry		Germination (%)
			Average	Percentage	
Autogamy	AGB002	80–85 (81.83 ± 1.94)	30–40 (33.33 ± 1.54)	80–87 (83.10 ± 3.90)	48–62 (56.66 ± 2.23)
Xenogamy					
	002 × 025 [+]	2–4* (3.59 ± 0.85)	9–14* (10.0 ± 1.57)	27–42 (30.22 ± 5.85)	15–27* (7.32 ± 4.84)
	025 × 002 [++]	4–9* (6.50 ± 0.84)	10–22* (14.33 ± 2.22)	28–62 (42.14 ± 10.52)	10–27* (15.21 ± 2.61)
	002 × 002 [+++]	6–13* (10.0 ± 1.06)	11–18* (14.00 ± 1.00)	33–54 (42.42 ± 4.79)	20–30* (25.66 ± 1.38)
Open pollination					
	AGB002	80–90 (86.66 ± 1.74)	28–44 (41.0 ± 2.69)	85–95 (87.30 ± 3.08)	55–65 (58.16 ± 1.64)

*Differences in per cent fruit set, seed set and germination between autogamy and xenogamy are significant ($P < 0.01$) by paired 't' test. Values in parenthesis are mean ± SE.
[+]110 flowers in 20 plants; [++]90 flowers in 20 plants; [+++]100 flowers in 20 plants.

42.14% ± 10.52 (for 025 × 002) and 42.42% ± 4.79 (for 002 × 002). Seeds obtained as a result of crossing also showed strikingly poor germination values about 8% (for 002 × 025), 16% (025 × 002) and 26% (for 002 × 002). In crossing e xperiments, it was noted that greater values were obtained for fruit set, seed set as well as germination when AGB002, i.e. the wild plant was the pollen source. Differences in per cent fruit set, seed set and germination between autogamy and xenogamy are significantly higher (Table 2).

The number of pollen grains differentiating per anther is profuse, averaging 5800 ± 175.7. Pollen grains are spherical or sub-spherical in shape. Ovules per ovary range between 29 and 53 (35.5 ± 5.85). Pollen to ovule ratio per flower averages 817 : 1. Pollen viability estimated by acetocarmine test was found to be high (90.5% ± 0.52) during peak flowering season (April–June). However, a reduction in pollen viability (80–83% by acetocarmine test; 65–70% by TTC test) was observed during the post-monsoon season (September). Similarly, in AGB-025 – a cultivated morphotype, pollen viability was recorded to be 83.5% ± 0.80 by acetocarmine test during peak flowering (February–May). During sporadic flowering in September, pollen viability exhibited reduction to the extent of 71–75% by acetocarmine test and 61–64% by TTC test. Pollen grains remain viable for 24 h in field conditions and 3–5 days when stored under low temperatures (10°C). The species bears scentless flowers with unattractive colour. Nevertheless, the flowers are visited by *Apis dorsata*, *Apis florea*, butterflies and some species of flies. The insect visit commences early in the morning, but is most active from 0800 to 1200 h.

High pollen load on the stigma and stiff pollen competition within a flower with about 817 pollen grains available for siring of one ovule, greatly reduce the possibility of cross-pollination. Small genetic differences existing among its morphotypes are maintained by high selfing rate. Hence it can be termed as a 'specialist' and not a 'generalist'. Similar observations are reported in *Plantago major*, termed as a specialist exhibiting a high degree of self compatibility[7,8]. The behaviour of insects on flowers and the insignificant pollen load carried by them further reduce the chances for cross-pollination. Similarly, seed-set percentages in autogamy and controlled cross-pollination (Table 2) also rule out any significant contribution of insects in the pollination of the species. Nevertheless, theoretically, the probability of a small degree of cross-pollination cannot be ruled out due to insect visits.

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