Gibberellins partly reverse the male sterile phenotype of tobacco

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MS-NPN-190 line of tobacco is male sterile. Its flowers possess stigmatoid stamens bearing ovules on carpelloid outgrowths. Administration of exogenous gibberellic acid (GA_3) inhibits the formation of ovules on the carpelloid outgrowths, but does not affect the stigmatoid distal end of the stamen. Male sterile stamens contain more sugars than wild type. Since the presence of high level of sugars is linked to endogenous gibberellins, our results suggest that MS-NPN-190 tobacco is not gibberellin-deficient. The suppression of formation of ovules on carpelloid branch in GA_3-treated stamens is possibly due to alteration in the balance among endogenous plant growth substances.

MALE sterility in flowering plants provides valuable information on molecular and developmental aspects of stamens and pollen grains. A review of the literature indicates the involvement of plant growth substances (PGSs) in male sterility and in normal stamen development[1,2]. To investigate the involvement of PGSs in male sterility, different approaches have been followed such as exogenous application of different PGSs, *in vitro* culture of normal and male sterile (ms) flower buds in media containing different PGSs, and estimation of levels of endogenous PGSs. Studies suggest that exogenous application of gibberellins promotes normal stamen development in gibberellin-deficient ms tomatoes[2–4]. Abscisic acid induces male sterility in wheat[5] and tomato[6]. Cytokinins and auxins stimulate the development of gynoecium and inhibit the development of stamens[2]. Ethylene is also known to promote gynoecium development and to induce male sterility[7,8]. Experiments have shown that gibberellins are important to restore fertility in ms mutants[3]. Although the molecular mechanism(s) by which plants respond to gibberellins are not fully understood, it is recognized that they induce the synthesis and secretion of a number of hydrolytic enzymes[9]. Exogenous application of gibberellin results in enhanced amylase activity and sugar content, and a decrease in starch in carpelloid stamens in gibberellin-deficient stamenless-2 mutant of tomato[10].

In MS-NPN-190 tobacco, stamens are converted into stigmatoid organs (Figure 1 *a* and *c*) with carpelloid outgrowths bearing external ovules (Figure 1 *d*). Thus, they resemble feminized stamens of ms mutant lines of tomato that can be converted into normal stamens by exogenous application of gibberellins[3,10,11]. In view of the above findings, the present work was taken up to determine whether or not an exogenous supply of gibberellic acid (GA_3) restores male fertility in MS-NPN-190 tobacco.

Seedlings of wild type and MS-NPN-190 mutant of tobacco were raised in identical conditions in the garden of the Department of Botany. To the seedlings bearing three-mature leaves, 10 µl of GA_3 solution at 10^{-2}, 10^{-3} and 10^{-4} M, in 0.02% (w/v) Tween-20, was applied in the axil of the leaf closest to the shoot apex, once in a five-day interval. Twenty-five plants were used for each concentration. Control seedlings were treated with 0.02% (w/v) Tween-20. Stamens were collected for the estimation of sugars[12] from the fully opened flowers of experimental and control plants. For anatomical studies, stamens were fixed in FAA, dehydrated in ethanol and butanol series and embedded in paraffin. Next, 5-µm thick microtome sections were stained with amidoblack-10B[13].

The experiments showed that exogenous application of GA_3 has no significant effect on the stamens of both the wild and mutant tobacco. At all concentrations of GA_3 used, the stigmatoid distal end of the stamen was not affected, although the formation of external ovules on the carpelloid outgrowths was suppressed (Figure 1 *b* and *e*). Estimation of sugars indicated higher concentration in ms stamens than in the wild type, which is reduced by GA_3 treatment (Figure 2). A similar effect of GA_3 was observed in the stamens

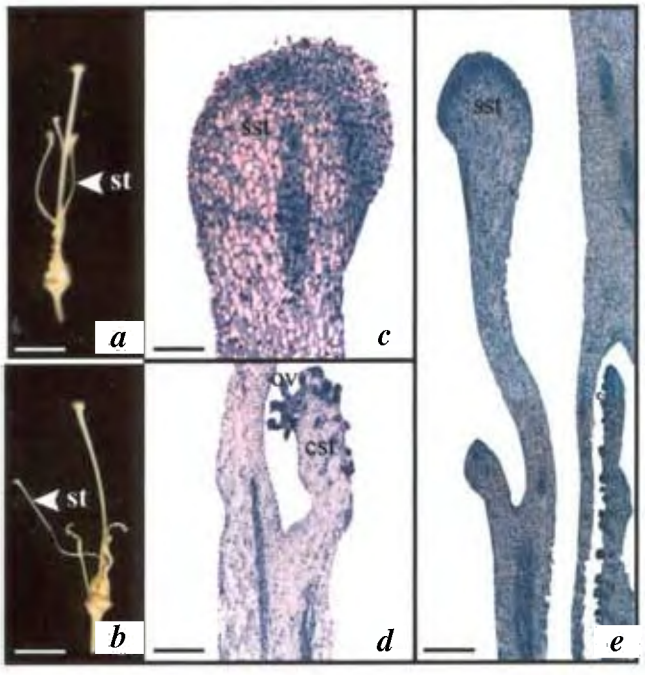

Figure 1. *a*, Whole mount of MS tobacco flower showing effeminate stamens (st); *b*, Feminized condition of stamens (st) is not altered in GA_3-treated ms tobacco; *c*, Stigmatoidal (sst) distal end of the stamen. *d*, Carpelloid outgrowth (cst) showing external ovules (ov). *e*, Inhibition of ovule formation on carpelloid outgrowth in GA_3-treated ms stamen. Stigmatoidal (sst) distal end is unaffected by GA_3-treatment. Bars: 16 µm (*c*); 50 µm (*d*); 60 µm (*e*).

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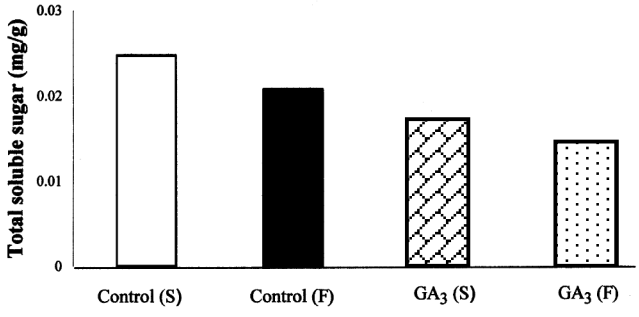

Figure 2. Level of sugar in mature stamens of control and GA_3-treated normal (F) and ms (S) flowers.

of wild type. Failure of exogenous supply of GA_3 to evoke any major response and the presence of high concentration of sugars in the stamens of both wild and ms mutant of tobacco possibly suggest that optimal levels of endogenous gibberellins are present in them. If MS-NPN-190 tobacco were to be gibberellin-deficient, due to impaired activity of amylases, their stamens should have contained less sugar than the wild type stamens. Therefore, the present results strongly suggest that MS-NPN-190 mutant of tobacco is not gibberellin-deficient. No effects of exogenous applications of GA_3 have been reported in other ms systems also[1]. On the contrary, stimulation of differentiation of carpelloid features on stamens by gibberellins has been observed in some hermaphrodite and monoecious plants[14]. The present results agree with the contention that gibberellins do not promote stamen development in ms plants, which are not gibberellin-deficient[1].

It is intriguing that the stamens of MS-NPN-190 tobacco contain more sugars than those in the wild type. One explanation can address this phenomenon. Earlier workers have estimated that maximum amylaceous reserves are consumed by the anther during the period from meiocyte stage to vacuolate microspore stage[15]. In ms stamens of tobacco, sugars are not consumed, apparently due to lack of growth and differentiation of anther. This probably explains why more sugars accumulate in ms stamens than in the wild type. Relatively low levels of sugars in GA_3-treated ms and wild type stamens may simply account for the increased quantitative growth of the stamens resulting from GA_3-treatment.

Analysis of endogenous PGSs in cucumber has shown higher gibberellin to auxin ratio in the andromonoecious line than in the gynoecious line[16,17]. Andromonoecious lines also produce less ethylene than the gynoecious lines[18]. These data suggest that male sterility is not related to either deficiency or overproduction of single PGSs and expression of sex organs involves a critical balance among several PGSs. This explains why application of GA_3 has a partial effect on the phenotype of MS-NPN-190 tobacco, as indicated by suppression of the formation of ovules on carpelloid outgrowths. In experimental plants exogenous application of GA_3 seemingly alters the balance of endogenous PGSs in such a way that the degree of 'femaleness' of stamens is partially, but not completely, reduced.

In conclusion, the effeminate condition of stamens of MS-NPN-190 tobacco is not a manifestation of endogenous levels of gibberellins. The higher level of sugars in ms stamens than in wild type not only suggests that MS-NPN-190 is not gibberellin-deficient, but also explains the failure of sugar metabolism in it. The suppression of formation of ovules on carpelloid outgrowths may be due to altered balance of endogenous PGSs, caused by the exogenous application of GA_3.

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Detection and monitoring of *Trichodesmium* blooms in the coastal waters off Saurashtra coast, India using IRS-P4 OCM data

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***Trichodesmium* blooms have been observed in the coastal waters off Saurashtra coast, Gujarat, India using Indian Remote Sensing satellite IRS-P4 Ocean Colour Monitor (OCM) data. Bloom-forming features were identified using false colour composites of channels 8, 6 and 5 (865, 670, 555 nm). Several bloom features of *Trichodesmium* like spiral eddies, stripes, slicks and parallel bands were detected in satellite images during 29 April to 7 May 2002. A strong backscattering signal was observed in the near-infrared band of OCM data, indicating the surface manifestation of *Trichodesmium* bloom. The chlorophyll images have been analysed during the bloom period; overestimation of pigments has been observed and the bloom patches appear darker. Subramaniam's protocol for *Trichodesmium* bloom study has been evaluated utilizing IRS-P4 OCM data for the waters off Saurashtra coast, and appeared to be relevant in detection by ocean colour remote sensing. The *in situ* information confirmed the presence of the bloom as coastal waters turned dark brown in colour with an appearance of sawdust spray.**

TRICHODESMIUM, a marine nitrogen-fixing cyanobacterium, forms extensive surface blooms that discolour vast regions of tropical and subtropical seas. *Trichodesmium* normally occurs in macroscopic bundles or colonies and is responsible for most of the N_2 fixation in oceanic and coastal waters[1]. It has been reported to bloom every year from February to May in near-shore waters off Goa as well as other locations along the west coast of India, where it mainly remains confined to the surface[2,3]. The mapping of *Trichodesmium* bloom has been carried out in the Coral Sea using CZCS data[4]. Infrared channels appear to show the potential for identifying *Trichodesmium* blooms. Surface cyanobacterial blooms have been observed with a distinct signal at 865 nm using SeaWiFS satellite data[5]. The presence of these blooms is reported to play an important role in the enrichment of water by releasing nitrogen and phosphorus. Nutrient enrichment assumes importance, since other processes of nutrient enrichment are minimal[6].

Trichodesmium can grow fast or bloom and accumulate as dense, visible patches near the surface of the water[7]. These algal blooms have been reported to promote the growth of diatoms and dinoflagellates on which the herbivores feed, followed by carnivores[8]. *Trichodesmium* has some unique characteristics that may help or hinder its detection by satellite data. In addition to its bloom-forming capacity, it has gas vacuoles that make it buoyant and keep it near the surface (within the upper 20 m), where colonies can be more readily detected[9]. *Trichodesmium* blooms are now of great scientific interest to the satellite remote sensing community. The phytoplankton in this bloom fixes nitrogen gas under fully aerobic conditions while photosynthetically evolving oxygen. It is now known to occur throughout the oligotrophic and subtropical oceans. The N_2 fixation property of *Trichodesmium* is likely to be a major input to the marine and global nitrogen cycle. Since fixed nitrogen commonly limits phytoplankton production in the ocean, oceanic nitrogen fixation has direct links to the ocean carbon cycle[10]. Stratification of the water column is a necessary condition for the formation of *Trichodesmium* blooms, as this allows cells to float on the surface with the help of their gas vacuoles. Stability of the surface waters is necessary for protection of the nitrogenase[11]. Although *Trichodesmium* is seen easily in the tropical seas, it has been difficult to detect it remotely by satellite. A robust detection protocol has been used[9]. But this is hampered by the fact that the spectral signature of *Trichodesmium* is strikingly identical to that of sediment plumes. High-resolution optical spectra at sea using hyper-spectral radiometers would resolve this problem[10].

However, the colour of the bloom varies and has been described as red, brown, green, yellow and silvery grey, depending on the age of the bloom[2] and the concentration of *Trichodesmium*[12]. In the present study we report the satellite detection and monitoring of *Trichodesmium* bloom in the coastal waters of the Arabian Sea along the Saurashtra coast, Gujarat, India. High spatial resolution data of Ocean Colour Monitor (OCM) sensor has been used for this study and satellite observations are supported by *in situ* investigations.

The study area lies in the coastal waters of the Arabian Sea along the west coast of India. The area is bounded by longitude 68.5 to 71.5°E and latitude 20 to 23°N. The

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