BREEDING OF MEDICINAL AND AROMATIC PLANTS (VOLUME - 2)

Editor's

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BREEDING OF SWEET FLAG

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Introduction

Sweet flag (*Acorus calamus*), a tall perennial wetland monocot plant from the Acoraceae family, is a well-known drug in traditional medicine. Its scented leaves and rhizomes are used as medicine. The plant exhibits significant chemical variations in essential oil and chromosome numbers, with a chromosome number of 12 and four natural cytotypes: diploid, triploid, tetraploid, and hexaploid.

The herb rarely produces seeds and is propagated by vegetative means. It is known for its fragrant aroma and medicinal properties. The rhizome has a spicy taste when dried and is an alternative to ginger, cinnamon, and nutmeg due to its odor. The leaves are simple, sword-shaped, and elongated, emerging from parallel to the ground rhizomes.

The genus Acorus includes approximately 40 different species, including *Acorus calamus*. It is widely distributed across India, both cultivated and natural habitats, and has been used for centuries. The *invitro* method of vegetative multiplication of *Acorus calamus* could have significant benefits for the medicinal trade and germplasm conservation. The rhizomes contain aromatic oil that has been used medicinally since ancient times and has been harvested commercially.

Parts Used: the leaves, roots (rhizomes), and stem of a plant, with rhizomes being the primary component in traditional medicine systems.

Botanical Description

- The herbaceous perennial *Acorus calamus* Linn. has a smooth, long, indefinitely branching rhizome that is pinkish or pale green in colour.
- It has brown, white, spongy leaf scars and fairly slender roots.

Dried Roots

Flowers

Roots

Berries

- There are just a few, sporadically alternating leaves, with an average of 1 cm in width and 0.7–1.7 cm in width. *Acorus calamus* has sympodial leaves that are shorter than vegetative leaves. The blooms are cylindrical, 3 to 8 cm long, greenish brown, and covered in a large number of spherical spikes.
- The fruits are discovered to be tiny, berry-like, and seed-free.
- American botanists traditionally assumed a single Acorus species, '*A. calamus*', was native to North America and identical to European *A. calamus*. However, European *A. calamus* were sterile and triploid, while most American Acorus populations produced seeds.
- Diploid plants, known as *A. americanus*, are widespread in North America, while sterile plants are confined to Eastern North America. Thompson (1993) found all 21 MD and 62 VA specimens were *Acorus calamus*.

Distribution

It may be found all across the tropics and subtropics but is most prevalent in Sri Lanka and India. It may be found in natural or farmed wetlands, rising as high as 1800 meters in Sikkim in the Himalayas. It is abundant in the Naga Hills, Manipur, and the marshy areas of Sirmoor and Kashmir. In the Karnataka taluk of Koratagere, it is commonly grown. The plant grows on light alluvial soil on riverbanks and clayey loams. Nowadays, it may be found in most English nations growing wild along the edges of pounds and rivers.

Varieties

The study aimed to identify a high essential oil yielding variety of *Acorus calamus*. Forty germplasm samples were collected from India and planted in RBD in 2014. After two seasons, a high essential oil genotype was identified as "Jor Lab AC-01". The elite genotype was evaluated in five locations in Northeast India in 2017, yielding an average of 1.20% more essential oil than the check variety. This new variety was suitable for commercial cultivation and farmers would benefit from its superior quality.

Popular Varieties with their Yield

1. ***Acorus calamus*:** Found in eastern North America and Eurasia. This variety's leaves are five feet long, with an elliptical spadix measuring four inches. The evergreen species have subterranean rhizomes that spread slowly.
2. ***Acorus calamus* Variegatus:** The leaves of this variety have cream and yellow color leaves.
3. ***Acorus gramineus* Argenteostriatus:** It is indigenous to Japan. This cultivar has around 3-inch-long blooms and leaves that grow 18 inches long. It grows slowly and has clusters that are two feet broad.
4. ***Acorus gramineus*:** Found in Pacific Northwest. It is evergreen plant.
5. ***Acorus gramineus* Golden Pheasant:** The foliage of this variety has golden to chartreuse color and 12-14 inches tall.
6. ***Acorus gramineus* Minimus Aureus:** The foliage of this variety has fine texture and is 4 inches tall.
7. ***Acorus gramineus* Ogon:** The variegated leaves of this variety have golden and green color which is 10-12 inches tall.
8. ***Acorus gramineus* Variegatus:** The variegated leaves have white and green color which is 8-12 inches tall.
9. ***Acorus gramineus* Yodo-No-Yuki:** This type has olive-green leaves with a hint of yellow around the edges. The leaves can reach a height of 12 inches.
10. ***Acorus gramineus* Hakuro-nishiki:** The foliage of this variety is yellowish green in color.

Ploidy level of *Acorus calamus* L

Population		Origin	Cytology	
Code	**State**	**Locality**	**Chromosome no: x =12**	**Ploidy**
A-44	Himachal Pradesh	Khaziar lake	2x = 24	Diploid
A-9	Himachal Pradesh	Shilly, Solan	3x = 36	Triploid
A-32	Himachal Pradesh	Sirmaur, Kotla	3x = 36	Triploid
A-33	Himachal Pradesh	Khaltoo, Solan	3x = 36	Triploid
A-42	Himachal Pradesh	Jatroon, Chamba	3x = 36	Triploid
A-34	Uttarakhand	Bhagwati, Almora	4x = 48	Tetraploid
A-35	Uttarakhand	Chaukhutiya, Almora	4x = 48	Tetraploid
A-36	Uttarakhand	Aagarchatti, Chamoli	4x = 48	Tetraploid
A-37	Uttarakhand	Dwarahat, Almora	4x = 48	Tetraploid
A-17	Jammu	Parol, Kathua	4x = 48	Tetraploid
A-18	Jammu	Jandi, Kathua	4x = 48	Tetraploid
A-19	Jammu	Khokharchak, Sambha	4x = 48	Tetraploid
A-3	Uttar Pradesh	NBRI, Lucknow	4x = 48	Tetraploid
A-4	Haryana	Hissar	4x = 48	Tetraploid
A-28	Arunachal Pradesh	Mao gate, Manipur	4x = 48	Tetraploid
A-51	Kashmir Valley	Check-i-Kawoosa	6x = 72	Hexaploid

Breeding Objectives

1. **Improved Oil Content:** Sweet flag contains essential oils with medicinal properties. Breeding for increased oil content can enhance its value in traditional medicine, aromatherapy, or the fragrance industry.
2. **Pest and Disease Resistance:** Developing varieties that are more resistant to pests and diseases can ensure better crop yields and reduce the need for pesticides or fungicides.
3. **High Yield Varieties:** Enhancing the plant's productivity to increase the yield per acre or area cultivated could be a key breeding goal, especially for commercial cultivation.
4. **Adaptation to Different Climates:** Breeding for adaptability to a wider range of climates can expand cultivation areas and make sweet flag more accessible to different regions.
5. **Improved Growth Characteristics:** Traits such as faster growth rates, better root development, or more vigorous growth can be targeted to make the plant easier to cultivate and manage.
6. **Consistent Chemical Composition:** Ensuring consistency in the concentration and composition of the bioactive compounds in sweet flag can be valuable, especially for medicinal or therapeutic purposes.
7. **Aesthetic Traits:** For ornamental purposes, breeding for specific aesthetic qualities like leaf color, size, or shape might be desirable.
8. **Reduced Toxin Content:** Sweet flag contains a compound called β-asarone, which in large quantities can be toxic. Developing varieties with reduced levels of this compound could improve the safety of its use.

Breeding Methods

S.No.	Breeding methods
1.	Selection
2.	Mutation
3.	Polyploidy
4.	Biotechnological approaches

Selection

The study analysed 29 sweet flag genotypes to assess genetic diversity, variability, heritability, and genetic advance. Six clusters were formed, with cluster III having the highest intra-cluster distance and the highest inter-cluster distance between clusters. High genotypic and phenotypic coefficients of variation were observed for various traits, suggesting greater variability among genotypes and responsiveness to selection. Moderate genetic variability and significant environmental influence were observed for plant height and leaf width.

Biotechnological Approaches

The study of Sweet Flag, a critically endangered species in India, used RAPD and ISSR markers to assess genetic variability among 20 accessions. Results showed 33.7% of bands were polymorphic, with Shannon's indices and Nei's genetic diversity estimated at 0.58, 0.57, and 0.40 respectively. The study suggests intraspecific variation studies could help develop conservation strategies for *Acorus calamus*.

- The study explores the impact of growth regulators on micropropagation of *Acorus calamus*, a rare plant in Iran used in health, food, and perfume industries. The study found that only the rhizome explant facilitated direct plant regeneration, while BAP and NAA treatments were effective for sweet flag regeneration. The Alandan population showed the highest root number in 1 mg/l treatment of IBA.
- The study identifies *Acorus calamus* L. accessions based on RAPD marker, ploidy level, and β-asarone content, which is crucial for medicinal use. Six primers generated polymorphisms, and genetic relatedness was evaluated. Accessions were found to be triploid and tetraploid, with β-asarone content in ranges of 6.92-8.0% and 73-88%. The findings could be useful in quality control of raw materials.
- The study evaluated the genetic diversity and population structure of 50 *Acorus calamus* populations in India using RAPD and chloroplast microsatellite markers. The results showed that 10 RAPD and nine cpSSR markers were polymorphic, with 65 polymorphic (67.70%) and 26 polymorphic (26 alleles) alleles. The mean genetic diversity among populations was higher than within populations. The mean coefficient of gene differentiation between populations was also high for both RAPD and cpSSR markers. AMOVA analysis revealed more genetic variation among populations than within populations. Significant differences were observed between populations and individuals within the populations. The study's findings could help devise strategies for the conservation of *Acorus calamus.*
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- The study aimed to identify genetic relationships in six different *Acorus calamus* L. populations using randomly amplified polymorphic DNA (RAPD) markers. The researchers amplified 574 DNA fragments using 10 primers, resulting in three major clusters: Lucknow and Paonta, Bangalore, and Solan, Nauni, and Hissar. The similarity coefficient values ranged from 0.97 to 0.88, with the highest coefficient (0.97) found between samples from Nauni and Hissar and Solan and Hissar, and

the lowest (0.88) between Lucknow and Bangalore, Lucknow and Solan, Bangalore and Solan. The low level of polymorphism indicated close relationships between samples.

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