

Indian calamus (*Acorus calamus* L.): not a tetraploid

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Acorus calamus Linn. (family Araceae), commonly known as sweet flag or 'Bach' in India, is an important medicinal and aromatic plant. In the present study, different accessions across the country were screened for ploidy status and possible correlation with β -asarone content. Most of the accessions were triploids with β -asarone content varying from 82.0 to 89.4% in their oil, except for only one triploid accession having 11.55% β -asarone content. Two diploid populations were also detected from western Himalayas which had low β -asarone contents of 11.67 and 7.39% respectively. The earlier notion that high β -asarone content in the Indian calamus was due to its tetraploid status does not hold true cytologically. Amongst phenotypic characters, lower specific leaf weight values, early autumn senescence and late emergence in spring in a population can be indicators for locating accessions with low β -asarone contents.

Keywords: *Acorus calamus*, calamus oil, β -asarone, ploidy status.

ACORUS CALAMUS Linn. (family Araceae), commonly known as sweet flag or 'Bach' in India, is an important medicinal and aromatic plant having wide usage in almost all herbal based systems. The rich ethnobotanical aspects of *A. calamus* have been reviewed¹⁻³. Based upon ploidy status and geographical distribution, *A. calamus* has been classified as (i) diploid variety ($2n = 2x = 24$; North America), (ii) triploid variety ($2n = 3x = 36$; Europe), (iii) the tetraploid variety ($2n = 4x = 48$; East Asia, India and Japan) and (iv) hexaploid variety ($2n = 6x = 72$; Kashmir area). The species has been primarily defined on the basis of genomic differences⁴. In the family Araceae as a whole, the basic chromosome number ranges from 8 to 22. The number 7 is suggested as the ancestral basic number of the family and it seems that aneuploid changes of chromosome number produced basic numbers of $x = 6, 7, 8, 9$ and 10 in early evolution of the family⁵. Previous reports of chromosome numbers in *A. calamus* have indicated 9, 11 and 12 as base numbers^{6,7}. Similarly, several chromosome counts have been reported for *A. calamus*, suggesting additional basic chromosome numbers, e.g., $x = 9$ ($2n = 45$ from South India⁵, $2n = 54$ from Kashmir⁸) and $x = 11$, or aneuploidy based on $x = 9$ ($2n = 44$ from

Thailand⁹). The chromosome count of $2n = 35$, is indicative for aneuploidy derived from the triploid cytotype with $2n = 36$ (ref. 10). Triploid *A. calamus* ($2n = 3x = 36$) has been recorded in some European countries indicative of 12 as the base number. Interestingly, the Kashmir population with $2n = 54$ (ref. 8) and $2n = 72$ (ref. 11) chromosomes have been designated as hexaploids.

The species appears to follow a geographical pattern of distribution with respect to ploidy level and the accessions growing in North America are diploid, whereas those growing in Europe and temperate Asia are primarily triploids and the ones growing in eastern and tropical Asia are tetraploids¹¹⁻¹³. Composition of the essential oil and particularly β -asarone content is reported to be dependent on the ploidy level of the taxon with a high range, i.e. 70–96% in tetraploids, low around 5–19% in triploids and zero in diploids^{14,15}. The β -asarone content in the Indian commercial calamus oil has been reported to be very high, i.e. more than 75% (refs 11–13), and thus it was assumed to be of tetraploid origin. The use of *Acorus calamus* with toxic β -asarone content has been reported to be unsafe on account of its tumour inducing activity¹⁶⁻¹⁸ and screening of different accessions for their asarone contents offers a solution to the problem^{11,18,19}.

In India, *A. calamus* grows in varying agroclimatic conditions right from the tropical south and subtropical plains to temperate marshes from Kashmir to the north east ascending to an altitude of 1500–2200 m in the Himalayan ranges. Keeping in view the conflicting ploidy status reported earlier, it was envisaged to revisit the ploidy status of Indian *A. calamus* and also the β -asarone content in different accessions across the country.

Accessions of *A. calamus* were procured from different representative locations in the country and were maintained under uniform cultivation conditions in the experimental farm of the Institute at Palampur (1300 m msl, 32.6°N and 78.18°E).

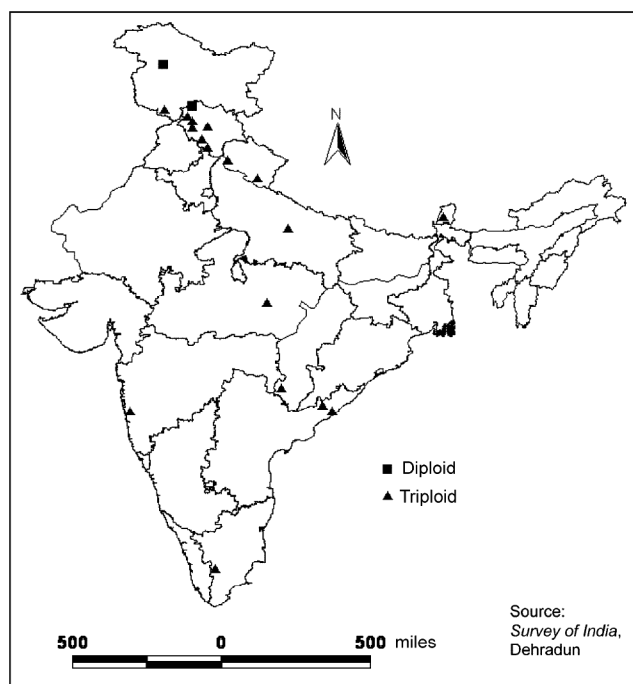
Actively growing young root tip segments were pre-treated with 8-hydroxy quinoline (2 mM) for 3½ h at 4°C and subsequently fixed in acetic acid:alcohol (1:3). These were hydrolysed in 1 N HCl at 60°C for 10–15 min and washed in distilled water prior to staining with Feulgen stain²⁰. The squash preparations were made in a drop of 2% acetocarmine. The temporary slides were observed under light microscope (Labophot, Nikon Corp., Japan) and photomicrographs were taken using digital camera (Nikon DXM1200, Nikon Corp., Japan).

The rhizomes of 14 accessions of *A. calamus*, collected from different locations (Table 1) were subjected to hydrodistillation in a Clevenger-type apparatus for 7 h. The oil obtained was dried over anhydrous sodium sulphate and stored in sealed glass vials in a refrigerator at 4°C. For analysing the percentage of β -asarone in calamus oil, gas chromatography (GC) analysis was conducted according to US Patent no. 6528041 (Sinha²¹) with

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Table 1. Relationship of ploidy status of Indian *Acorus calamus* accessions with β -asarone content

Accession no.	Altitude (m amsl)	Ploidy status		β -asarone content (%)	Oil yield (%) (fresh weight basis)	SLW (mg/cm ²)
		Diploid $2n = 2x = 24$	Triploid $2n = 3x = 36$			
IHB/BT/02-35, P-3/04-03	1000	—	✓	86.01	1.18	25.33
IHB/BT/02-04, P-3/04-03	650	—	✓	89.40	1.82	25.30
IHB/BT/02-69, P-3/04-03	1220	—	✓	88.43	0.99	26.66
IHB/BT/02-97, P-3/04-03	1500	—	✓	84.13	1.31	27.00
IHB/BT/02-104, P-3/04-03	1400	—	✓	88.75	2.50	27.90
IHB/BT/02-46, P-3/04-03	500	—	✓	82.00	0.85	31.00
IHB/BT/02-67, P-3/09-03	103	—	✓	85.15	1.76	27.45
IHB/BT/02-63, P-3/09-03	183	—	✓	87.16	1.52	29.35
IHB/BT/02-64, P-3/08-03	700	—	✓	85.37	1.21	30.73
IHB/BT/02-10, P-3/04-03	1600	✓	—	11.67	0.42	21.80
IHB/BT/02-88, P-3/05-03	1280	—	✓	86.97	1.13	30.13
IHB/BT/02-75, P-3/04-03	600	—	✓	84.21	0.64	28.12
IHB/BT/02-80, P-3/04-03	600	—	✓	11.55	0.73	28.73
IHB/BT/02-73, P-3/09-06	1900	✓	—	07.39	0.58	22.50

**Figure 1.** Distribution of diploids and triploids of Indian *Acorus calamus*.

the following conditions: SE-30 column, 30 m \times 0.25 mm; injector temp. 250°C; flame ionization detector 230°C; programmed for 40 min (hold for 2 min) to 220°C (hold for 10 min) 10°C per minute; injection volume 1 μ l; N₂ flows 30 ml/min; split injection ratio 1 : 30.

Specific leaf weight (SLW) was calculated according to Chairiello *et al.*²². Leaf discs (1.0 cm) from third fully expanded leaf were carved by a borer at a uniform distance of 20 cm from the tip avoiding midrib area. Three replications (three leaf discs per replication) were main-

tained for each sample. The samples were dried to a constant weight at 70°C and SLW expressed as mg/cm².

In the present study, most of the tropical and subtropical accessions collected from different geographical locations of India showed $2n = 3x = 36$, whereas only two accessions collected from temperate western Himalaya had $2n = 2x = 24$ chromosomes (Table 1; Figure 1). The ploidy status in *A. calamus* is known to affect the qualitative and quantitative composition of its essential oil. The different accessions under study varied in their oil yield and β -asarone contents. The major constituent of calamus oil is β -asarone. The oil composition in *A. calamus* is greatly affected by its geographical location and growth stage^{11,13}. Thus accessions with high oil yields had high β -asarone contents, whereas those having low β -asarone also showed very low oil yields (Table 1). The β -asarone content in most of the accessions was very high (82.0 to 89.4%) except for the only one triploid accession (Figure 2 a) with very low 11.55% β -asarone content. The two diploid populations ($2n = 2x = 24$; Figure 2 b and c) had very low, i.e. 11.67% and 7.39% β -asarone content respectively as compared to the majority of the triploid accessions presently investigated. The relative pattern of β -asarone content in relation to the ploidy status of the Indian *A. calamus* is depicted in Figure 3. The low levels of β -asarone in these accessions are, however, comparable to the triploids of European origin. Janaki Ammal *et al.*⁸ have reported that the accession from Kashmir yields a lighter oil (sp. gr. 0.97) as compared to the oil obtained from accession collected from Jammu region (sp. gr. 1.05), indicating the chemotypic similarity of Kashmir *A. calamus* with the European one. Both also have low β -asarone contents irrespective of their different ploidy levels. β -asarone has been reported to be absent in some diploid varieties²³, whereas in triploids and tetraploids it constitutes up to 5% (ref. 11) and around 96% (ref. 14) respec-

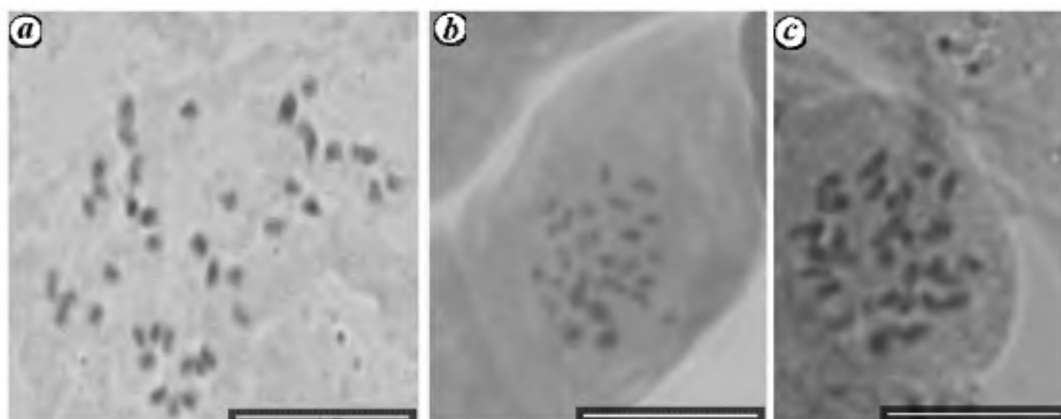


Figure 2. Cytology of screened low β -asarone accessions of Indian *Acorus calamus*. **a**, IHB/BT/02-80, P-3/04-03, $2n = 3x = 36$; **b**, IHB/BT/02-10, P-3/04-03, $2n = 2x = 24$; **c**, IHB/BT/02-73, P-3,09-06, $2n = 2x = 24$; Bar = 10 μ m.

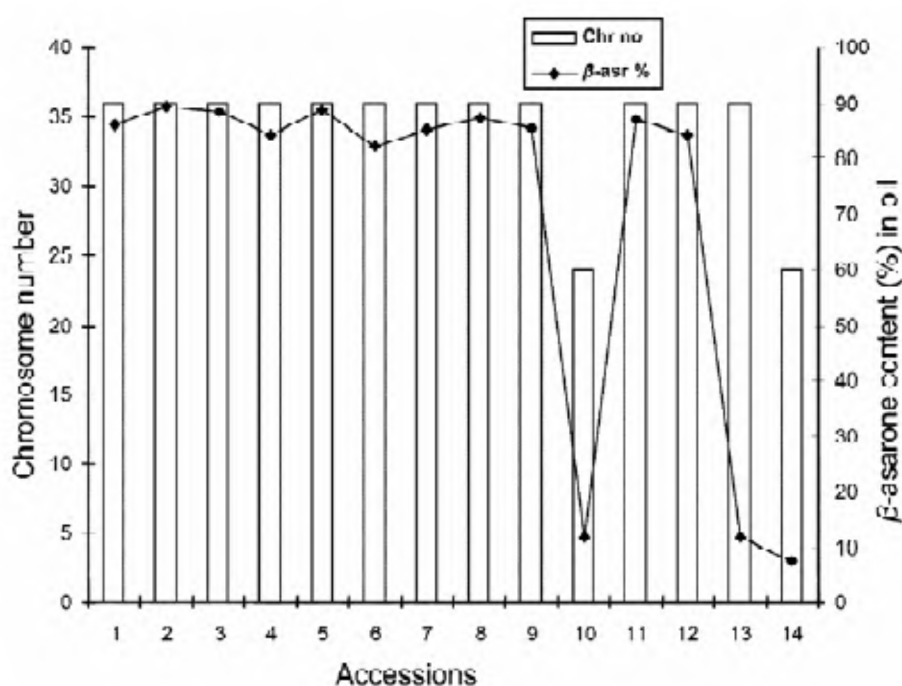


Figure 3. Relative correlation of ploidy status and β -asarone content in some Indian *Acorus calamus* accessions.

tively. The high β -asarone content in Indian *A. calamus* has therefore led to the notion that Indian calamus is tetraploid. However, the bulk of Indian *A. calamus* was found to be triploid instead of tetraploid (Figure 1). Röst and Bos¹⁴ have also shown that *A. calamus* has several chemodemes with respect to the composition of essential oils and the different cytotypes, e.g. diploid, triploid and tetraploid could not be unambiguously characterized by individual oil composition. This is evident from the fact that whereas the triploids of Europe are characterized by low β -asarone contents, i.e. 9–20%, the triploids collected from lower Himalaya and Indian subcontinent have very high β -asarone contents, which is comparable to the tetraploids reported in nature. The assumption by Mazza¹¹ and Raina *et al.*¹³ that origin of Indian commercial cala-

mus oil is from tetraploids therefore does not hold true, as in the present survey mostly triploids are reported with high β -asarone contents.

The different accessions showed variability in the SLW. The range varied from 21.8 to 31.0 mg/cm². The diploid accessions had much lower SLW (approximately 22.0 mg/cm²) as compared to triploids (more than 27 mg/cm²). Hence SLW could also be used to characterize the diploids in nature. Earlier, various characters like leaf length, leaf width, leaf apex, stomatal size, spadix diameter, angle, etc. have been studied in the biosystematic evaluation of *Acorus*⁴, but none of these were found to be representative of the ploidy level. SLW, an index of leaf thickness, varies considerably with different cytotypes of *Acorus* which is corroborated by the obser-

vation of Röst⁴ that air canals per unit area in the central part of the blades is the only reliable structural character for identification of cytotypes in *Acorus*. A remarkable feature of early autumn senescence and late spring growth was found in all the three accessions with low β -asarone contents irrespective of their ploidy levels, as compared to other accessions with high β -asarone contents growing under identical conditions at our campus.

In conclusion, most of the accessions from Indian sub-continent were predominantly triploids with very high β -asarone contents and not tetraploids as reported earlier. Only one triploid accession was found to be with low β -asarone content. Two diploids were also detected with very low β -asarone contents from temperate western Himalayas. All the low β -asarone accessions were collected from western Himalayas and appear to be more akin to European *A. calamus* with respect to β -asarone contents. Amongst the phenotypic characters, lower SLW values and quicker senescence and late emergence in a population may help in preliminary quick screening of accessions for low β -asarone contents.

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ACKNOWLEDGEMENTS. The financial assistance from Department of Biotechnology, Govt of India is gratefully acknowledged.

Received 11 February 2009; revised accepted 22 September 2009

Mature coconut as a bio-fermentor for multiplication of plant growth promoting rhizobacteria

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A cheap and farmer-friendly multiplication method for plant growth promoting rhizobacteria (PGPR) in mature coconut is described here. Uncontaminated coconut water naturally available within the coconut was used as medium for multiplication of two PGPR strains, *Pseudomonas* sp. PN026R and *Bacillus pumilus* SE34. Bacterial strains were separately inoculated in aseptically collected coconut water as well as coconut water in mature intact coconut and the growth pattern was studied. Growth of PGPR strains with coconut water as a sole nutrient source was comparable to that in conventional liquid medium. Both the strains multiplied in the coconut water to the tune of 10^8 cfu ml⁻¹ within a period of 24 h. The bacterial strains developed mucoidal colonies on coconut water agar medium as a result of increased polysaccharide production. Seed colonization and plant growth pro-

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