Molecular characterization of ten cultivars of Canna lilies (Canna Linn.) using PCR based molecular markers (RAPDs and ISSRs)

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Abstract
Molecular markers like RAPD and ISSR were used to study the genomic affinity among 10 cultivars of Canna lilies (Canna linn.). 15 numbers of decamer oligonucleotide primers produced a total of 103 bands out of which 20 were monomorphic and among the polymorphic bands there were 16 unique bands. Three ISSR primers produced 27 bands among which there were 21 markers and one cultivar specific band. The dendrogram constructed on the basis of both RAPD as well as the ISSR data showed two clusters. Canna x generalis “Lucifer” was isolated from rest of the varieties. All the other nine varieties were again sub-divided to two categories. Canna x generalis “Scarlet” was separated out and the varieties Canna x indica “Tropicanna” and Canna indica were grouped under a single sub-cluster sharing a common node at 56% similarity level. Jaccard’s similarity derived from the common data revealed that Canna x generalis “Richard Wallace” and “Rosamond” had maximum similarity with similarity coefficient of 0.646 and the varieties “Lucifer” had maximum distance from “Purpurea” with similarity value of 0.402. All the varieties were related with each other with an average similarity value of 0.540. The cophenetic correlation showed that the variety Canna x generalis “President and “Grumpy” have close affinity to each other with a similarity value of 0.644 and Canna x generalis “Lucifer” maintained an equal distance from all other varieties with a similarity coefficient of 0.472. Though RAPD and ISSR method can not delimit the species and hybrids but can provide useful information for breeding programme.

INTRODUCTION
The genus Canna is comprised of only 8-10 wild species and over 1,000 hybrids which are used as cultivated garden ornamentals in Europe, North America, and several tropical parts of the world. Although the cannas were previously considered as simple foliage plants, during the last two centuries of cultivation and improvement they transformed them into attractive ornamental flowering plants with reduction in plant height, increase in hardiness, variability in flower colours and such other positive attributes. Hundreds of hybrids have evolved from complex crosses between various species of Canna, they are often grouped under the names C. x generalis L. H. Bailey and C. x orchioides L. H. Bailey. As the distinction between these hybrid groups have been blurred by further inter-breeding involving parents from one or more than one species, varieties and hybrids. Canna x generalis (C. glauca x C. indica x C. iridiflora x C. warscewiczii) and C. x orchioides (C. glauca x C. indica x C. iridiflora x C. warscewiczii x C. flaccida) are horticultural species under which all the ornamental cultivars and hybrids were included. The elemental species, on improvement to garden varieties, changed greatly, i.e spike come out well above the foliage, free flowering habit, color diversity and durability, size and thickness of the flowers, thereby making Canna as colourful garden
Genetic diversity in *Canna generalis*

Table 1: Morphological information of different cultivars of Canna.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of the species/ cultivar</th>
<th>Plant height</th>
<th>Flower colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Var1</td>
<td>Canna x generalis “Lucifer”</td>
<td>60-75 cm</td>
<td>Orange, with yellow-mottled petal margins, base and eye.</td>
</tr>
<tr>
<td>Var2</td>
<td>Canna x generalis “President”</td>
<td>100-120 cm</td>
<td>Red, with a thin yellow margin along the border of the petals and yellow specks on base of petals.</td>
</tr>
<tr>
<td>Var3</td>
<td>Canna x generalis “Richard Wallace”</td>
<td>80-100 cm</td>
<td>Yellow, sometimes with orange spots towards the base of the petals.</td>
</tr>
<tr>
<td>Var4</td>
<td>Canna x generalis “Purpurea”</td>
<td>45-55 cm</td>
<td>Pink, deep pink towards the throat of corolla.</td>
</tr>
<tr>
<td>Var5</td>
<td>Canna x generalis “Grumpty”</td>
<td>100-110 cm</td>
<td>Crimson red, sometime with a maroon border along the petal margin.</td>
</tr>
<tr>
<td>Var6</td>
<td>Canna x generalis “King Humbert”</td>
<td>70-85 cm</td>
<td>Deep red, sometime few yellow lines/patches on the middle portion of the petals.</td>
</tr>
<tr>
<td>Var7</td>
<td>Canna x generalis “Scarlet Beauty”</td>
<td>90-100 cm</td>
<td>Orange, yellow towards the tip of the petals.</td>
</tr>
<tr>
<td>Var8</td>
<td>Canna x generalis “Rosamond”</td>
<td>100-150 cm</td>
<td>Orange</td>
</tr>
<tr>
<td>Var9</td>
<td>Canna x indica “Tropicanna”</td>
<td>80-100 cm</td>
<td>Orange</td>
</tr>
<tr>
<td>Var10</td>
<td>Canna indica</td>
<td>100-150 cm</td>
<td>Red</td>
</tr>
</tbody>
</table>

There is no published literature on assessment of genetic diversity in Canna lilies using molecular techniques. Taking the following characters into consideration, the complex nature of origin of different species, cultivars and hybrids and wide ranging variability in Cannas with regard to flower colour, plant height, foliage and such other variable characters, the present investigation was taken up to genetically characterize 10 common and popular cultivars of *Canna x generalis* and *C. indica* using molecular markers like RAPD and ISSR.

**MATERIALS AND METHODS**

**Plant materials**

Ten different selected cultivars of *Canna* belonging to *C. x generalis* and *C. indica* complex were collected (Table-1) from the Botanic Gardens of the Regional Plant Resource Center (RPRC), Bhubaneswar, Orissa, India, which were originally collected from different commercial nurseries of the country and maintained under controlled climatic conditions in green-houses.

**Isolation of Genomic DNA**

DNA was isolated from young and fresh leaves using the CTAB method as described by Saghai-Marroof *et al.* (1984). RNA were removed by giving RNaseA treatment (@ 60μg for 1ml of crude DNA solution at 37°C) followed by two washes of Phenol: Chloroform:Iso-Amyl-Alchol (25:24:1) and subsequently two washes with Chloroform:Iso-Amyl-Alchol (24:1). After centrifugation the upper aqueous phase was separated, 1/10 volume 3M-sodium acetate (pH 4.8) was added and DNA was precipitated with 2.5 volume of pre chilled absolute ethanol. DNA was dried and dissolved in T10E1 buffer (Tris-Cl 10mM, EDTA 1mM pH 8). Quantification was made by running the dissolved DNA 1 n 0.8% agarose gel along side uncut λ DNA of known concentration. The DNA was diluted to 25ng per μl for RAPD and ISSR analysis.

**RAPD analysis**

For RAPD analysis, PCR amplification of 25 ng of genomic DNA was carried out using standard 22 decamer oligonucleotide primers, out of
Genetic diversity in *Canna generalis*

Table 2: Details of RAPD and ISSR analysis of 10 Cultivars of Canna

<table>
<thead>
<tr>
<th>Primer</th>
<th>Range of amplicons (Bp)</th>
<th>Total No. of Bands</th>
<th>No. of Polymorphic Bands</th>
<th>No. of Monomorphic Bands</th>
<th>No. of Unique</th>
<th>Resolving Power</th>
<th>RAPD / ISSR primer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPA02</td>
<td>2000-500</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6.1</td>
<td>1</td>
</tr>
<tr>
<td>OPA03</td>
<td>1800-500</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>8.6</td>
<td>10.4</td>
</tr>
<tr>
<td>OPA04</td>
<td>2400-200</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>5</td>
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<td>OPA10</td>
<td>3000-600</td>
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<td>10.8</td>
</tr>
<tr>
<td>OPA18</td>
<td>1800-1100</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3.6</td>
<td>2.4</td>
</tr>
<tr>
<td>OPC02</td>
<td>1600-800</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>7.6</td>
<td>6.4</td>
</tr>
<tr>
<td>OPC05</td>
<td>3000-1031</td>
<td>8</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>8.2</td>
<td>7.8</td>
</tr>
<tr>
<td>OPC12</td>
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<td>3</td>
<td>0</td>
<td>0</td>
<td>3.2</td>
<td>2.8</td>
</tr>
<tr>
<td>OPD02</td>
<td>2500-400</td>
<td>11</td>
<td>9</td>
<td>2</td>
<td>3</td>
<td>9</td>
<td>13</td>
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<tr>
<td>OPD03</td>
<td>2000-750</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>6.2</td>
<td>1.8</td>
</tr>
<tr>
<td>OPD08</td>
<td>1700-350</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>9.8</td>
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<tr>
<td>OPD16</td>
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<td>6</td>
<td>1</td>
<td>4</td>
<td>3.6</td>
<td>10.4</td>
</tr>
<tr>
<td>OPN4</td>
<td>1800-600</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>8.4</td>
<td>1.6</td>
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<tr>
<td>OPN05</td>
<td>2200-550</td>
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<td>OPN6</td>
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<tr>
<td>(AGG)6</td>
<td>2900-450</td>
<td>11</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>9.4</td>
<td>12.6</td>
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<tr>
<td>(GACA)4</td>
<td>2000-600</td>
<td>8</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>11.4</td>
<td>4.6</td>
</tr>
<tr>
<td>T(GA)9</td>
<td>2000-500</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>6.6</td>
<td>9.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2000-500</strong></td>
<td><strong>6</strong></td>
<td><strong>6</strong></td>
<td><strong>0</strong></td>
<td><strong>0</strong></td>
<td><strong>6.1</strong></td>
<td><strong>1</strong></td>
</tr>
</tbody>
</table>

which 15 primers was found to be amplified repeatably. So in the present investigation primers of A, C, D and N series (OPA02, OPA03, OPA04, OPA10, OPA18, OPC02, OPC05, OPC12, OPD02, OPD03, OPD08, OPD16, OPN04, OPN05 and OPN06; Operon Tech. Alameda, CA, USA) were used for RAPD analysis. The RAPD analysis was performed as per the standard methods of Williams et al. (1990). Each amplification reaction mixture of 25μl contained 20ng of template DNA, 2.5μl of 10X assay buffer (100mM Tris-HCl pH8.3, 0.5M KCl and 0.01% Gelatin), 1.5mM MgCl₂, 200μm each of dNTPs, 20 ng of primer and 0.5U Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). The amplification was carried out in a thermal cycler (GeneAmp PCR System 9700, Applied Biosystems, CA, USA). The first cycle consisted of denaturation of template DNA at 94°C for 5 min, primer annealing at 37 °C for 1 min and primer extension at 72 °C for 2min. In the subsequent 42 cycles the period of denaturation was reduced to 1min while the primer annealing and primer extension time was maintained the same as in the first cycle. The last cycle consisted of only primer extension at 72 °C for 7 min. PCR products were separated on a 1.5% agarose gel containing ethidium bromide solution (@0.5μg/ml of gel solution). The size of the amplicons was determined using size standards (100bp ladder plus or DNA ladder mix, MBI Fermentas, Lithuania). DNA fragments were visualized under UV light, documented in Gel Doc (Bio-Rad, USA) and photographed.
ISSR analysis

Inter-simple sequence repeats has recently been developed which access the variation in the numerous microsatellite regions distributed throughout different genomes (basically the nuclear genome) and bypass the challenges of characterizing individual loci that other molecular techniques require. Three selected simple sequence repeats (Table-2) (Bangalore Genei Pvt. Ltd., Bangalore, India) were used for PCR amplification.

Each amplification reaction mixture of 25μl contained 20ng of template DNA, 2.5μl of 10X assay buffer (100mM Tris-HCl pH8.3, 0.5M KCl and 0.01% Gelatin), 1.5mM MgCl2, 200μm each of dNTPs, 44ng of primer and 0.5U Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). The amplification was carried out in a thermal cycler (GeneAmp PCR System 9700, Applied Biosystems, CA, USA). The first cycle consisted of denaturation of template DNA at 94 °C for 5 min, primer annealing at 50-55 °C for 1 min and primer extension at 72 °C for 2min. In the subsequent 42 cycles the period of denaturation was reduced to 1min while the primer annealing and primer extension time was maintained the same as in the first cycle. The last cycle consisted of only primer extension at 72 °C for 7 min. PCR products were separated on a 2 % agarose gel containing ethidium bromide solution (@0.5μg/ml of gel solution). The size of the ampiclons was determined using size standards (100bp ladder plus or DNA ladder mix, MBI Fermentas, Lithuania). DNA fragments were visualized under UV light, documented in Gel Doc (Bio-Rad, USA) and photographed.

Data analysis

The bands amplified from RAPD and ISSR were scored as '1' and '0' for presence and absence of band respectively. All the bands whether monomorphic or polymorphic were used for similarity calculation in order to avoid over estimation of distance (Gherardi et al., 1998). Jaccard's coefficient of similarity (Jaccard, 1908) was calculated and a dendrogram based on similarity coefficient was obtained through un-weighted pair group method using arithmetic averages (UPGMA)(Sneth and Sokal, 1973) and SHAN clustering. All the analysis were done by using the computer package NTSYS-PC-2.02e (Rohlf, 1997). Resolving power of the RAPD and ISSR primers were calculated as per Prevost and Wilkinson (1999). Resolving power is: Rp =ΣIB (IB (Band informative ness)= 1- [2×(0.5-P)], P is the proportion of the 10 species containing the band. RAPD and ISSR Primer Index. The primer index was calculated from the polymorphic index. A polymorphic index (PIC) was calculated as PIC = 1-ΣP², P, is the band frequency of the i-th allele (Smith et al. 1997). In the case of RAPDs and ISSRs, the PIC was considered to be 1-p²-q², where p is band frequency and q is no band frequency (Ghislain et al. 1999). PIC value was then used to calculate the RAPD primer index (RPI). RPI is the sum of the PIC of all the markers amplified by the same primer. Principal co-ordinate analysis (PCA) was used to retrieve information about the clustering pattern of the analyzed populations. PCA was performed based on the RAPD and ISSR data, for all the primers.

RESULTS

Random Amplified Polymorphic DNA (RAPD) analysis

Twenty two random decamer oligonucleotide primers were used for RAPD analysis. Out of these seven primers did not give satisfactory amplification. The 15 primers namely OPA02, OPA03, OPA04, OPA10, OPA18, OPC02, OPC05, OPC12, OPD02, OPD03, OPD08, OPD16, OPN04, OPN05 and OPN06 responded well and gave very good amplification. The RAPD banding pattern is represented in Fig.-1. All the primers gave wide range of fragments ranging from 100bp to 3000bp. The highest number of fragments (10) were amplified by the primer OPA10 and that was lowest by the primer OPC12 and OPA18 (3). The primers OPN05 yielded maximum number of 10 polymorphic bands and the number of polymorphic bands was lowest in case of OPN04 (1). It was interesting to note that maximum number of monomorphic bands and the number of polymorphic bands was lowest in case of OPN04 (1). The RPI was calculated from the polymorphic index. The Resolving Power (RP) was highest in case of OPD08 (9.8) and RAPD primer index (RPI) was highest in case of OPD02 (13) whereas the lowest RP was observed for OPC12 (3.2) and the lowest RPI
Genetic diversity in *Canna generalis*

was observed in OPA02 (1). The dendrogram constructed by SHAN clustering using Jaccard’s similarity coefficient showed two distinct clusters containing 3 and 7 cultivars. In the small clade *Canna x generalis* “Scarlet Beauty” and “Lucifer” were grouped with *Canna x indica* “Tropicanna” while in the other cluster *Canna indica* was grouped along with *Canna x generalis* “President”, “Grumpty”, “Richard Wallace”, “Rosamond”, “Purpurea” and “King Humbert” (Figure-2). Both the clusters shared a common node at 50 % level of similarity. The Jaccard’s similarity shows that the cultivar *Canna x generalis* “Richard Wallace” and “Rosamond” were most closely related with a similarity value of 0.765 and the variety *Canna generalis* “Canna x indica” with a similarity value of 0.546.

**Inter Simple Sequence Repeat (ISSR) analysis**

Three ISSR primers yielded 27 bands out of which 22 were polymorphic and only five bands were monomorphic in nature. Among these polymorphic bands only two bands were unique. The amplicons were in the range of 2500 to 450 base pairs. The resolving power for the three primers (GACA)$_4$, (AGG)$_6$, and T(GA)$_9$ were 11.4, 9.4 and 6.6 respectively and the primer index for these primers were 4.6, 12.6 and 9.4 respectively (Table-2). The dendrogram constructed on the basis of SHAN clustering using Jaccard’s similarity showed two major clades containing three and seven cultivars. Both the clusters shared a common node at 40 % level of similarity (Figure not shown). In the dendrogram *Canna X generalis* “Lucifer” was grouped along with *Canna x indica* “Tropicanna” and *Canna indica*. Jaccard’s similarity coefficient showed that the variety *Canna x generalis* “Scarlet Beauty” and *Canna x generalis* “Rosamond” were very closely related with a similarity value of 0.765 and the variety *Canna X generalis* “Rosamond” and *Canna indica* were widely apart with similarity value of 0.304 (Table-4 [Supplementary data]). All the individuals were related with each other with an average similarity of 0.509.
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When both the markers were analyzed in conjunction a different picture was obtained. The dendrogram constructed on the basis of both RAPD as well as the ISSR data showed two clusters. *Canna x generalis* “Lucifer” was isolated from rest of the varieties (Figure-3). All the other nine varieties were again sub-divided to two categories. *Canna x generalis* “Scarlet” was separated out and the varieties *Canna x indica* “Tropicanna” and *Canna indica* were grouped under a single sub-cluster sharing a common node at 56% similarity level. Jaccards similarity derived from the common data revealed that *Canna x generalis* “Richard Wallace” and “Rosamond” had maximum similarity with similarity coefficient of 0.646 and the varieties “Lucifer” had maximum distance from “Purpurea” with similarity value of 0.402. (Table-5 [Supplementary data]). All the varieties were related with each other with an average similarity value of 0.540. The cophenetic correlation showed that the variety *Canna x*

![Figure 2: Dendrogram showing genetic relationship among 10 cultivars of Canna based on RAPD.](image1)

![Figure 3: Dendrogram showing genetic relationship among 10 cultivars of Canna based on RAPD and ISSR.](image2)

**Analysis of RAPD and ISSR data**

When both the markers were analyzed in conjunction a different picture was obtained. The dendrogram constructed on the basis of both RAPD as well as the ISSR data showed two clusters. *Canna x generalis* “Lucifer” was isolated from rest of the varieties (Figure-3). All the other nine varieties were again sub-divided to two categories. *Canna x generalis* “Scarlet” was separated out and the varieties *Canna x indica* “Tropicanna” and *Canna indica* were grouped under a single sub-cluster sharing a common node at 56% similarity level. Jaccards similarity derived from the common data revealed that *Canna x generalis* “Richard Wallace” and “Rosamond” had maximum similarity with similarity coefficient of 0.646 and the varieties “Lucifer” had maximum distance from “Purpurea” with similarity value of 0.402. (Table-5 [Supplementary data]). All the varieties were related with each other with an average similarity value of 0.540. The cophenetic correlation showed that the variety *Canna x*
Genetic diversity in *Canna generalis*

“President and “Grumpty” have close affinity to each other with a similarity value of 0.644 and *Canna X generalis “Lucifer”* maintained an equal distance from all other varieties with a similarity coefficient of 0.472 (Table-6 [Supplementary data]). The cophenetic correlation showed all the varieties were genetically related to each other with an average similarity value of 0.5407.

**Correlation among markers**

Correlation among similarity matrix of combined data with that of RAPD showed a high degree of similarity with ‘r’ value 0.90. But the ‘r’ value among similarity matrix of combined data with that of ISSR was 0.52. This indicated that the combined data was much more similar with that of the data obtained from RAPD analysis.

**Principal Coordinate Analysis (PCA)**

The PCA analysis showed that all the cultivars were intermingled (Fig.- 4). All the varieties of *Canna x generalis* and *Canna indica* were found together. The variety *Canna x generalis “Purpurea”* was isolated from other cultivars being placed at a distant corner.

![Figure 4: Principal co-ordinate analysis of the 10 cultivars of Cannas](image)

**DISCUSSION**

Quite considerable genetic variability does exist among different species, varieties and hybrids of *Canna* cultivated in Indian gardens. There is no published report on assessment of genetic diversity in cultivated *Canna* lilies in India and elsewhere except the one by Prince & Kress (2001). They used nuclear ITS and chloroplast rpl16 intron DNA sequence data for 22 plants representing 7 broadly-defined species of *Canna* and the molecular data confirmed the recognition of a limited number of species including a broadly defined *Canna indica*. In view of lack of information in the area of molecular characterization of genetic variability in cultivated Cannas in Indian gardens, the present study was initiated involving 10 common cultivars belonging to the horticultural species *Canna x generalis* and the elemental species *Canna indica*. Molecular markers like RAPD and ISSR were used to assess the genome relationship among these 10 cultivated forms of *Canna*.

For RAPD analysis, twenty two random decamer oligonucleotide primers were used. The dendrogram constructed by pooled RAPD data showed two distinct clusters containing 3 and 7 cultivars. In the small clad *Canna x generalis “Scarlet Beauty”* and “Lucifer” were grouped with *Canna x indica “Tropicanna”* while in the other cluster *Canna indica* was grouped along with *Canna x generalis “President”, “Grumpty”, “Richard Wallace”, “Rosamond”, “Purpurea” and “King Humbert*. Both the clusters shared a common node at 50 % level of similarity. It was observed that the cultivar *Canna x generalis “Richard Wallace”* and “Rosamond” were most closely related with a similarity value of 0.661 and the variety *Canna x generalis “Purpurea”* was remotely placed in relation to *Canna x generalis “Lucifer”* with the similarity value of 0.414 (Table-3 [Supplementary data]). However, each cultivar had an average similarity of 0.546 with each other cultivar in the group.

Rajaseger *et al.* (1997) analyzed the genetic diversity among 22 cultivars of *Ixora* through RAPD and found DNA markers useful in distinguishing closely related cultivars. All 22 cultivars distinctly grouped under two cultivar groups, viz., *Ixora coccinea* and *I. javanica*. The study indicated that besides the use of RAPD markers for identification of particular *Ixora* cultivars, the phylogenetic relationships of
generated by RAPD analysis might be useful for varietal improvement programmes. However, in
the present study it was observed that though considerable genetic differences exist between
and among cultivars of Canna, it was not possible to segregate the horticultural species Canna x generalis and its hybrids from the
elemental species C. indica and its only cultivar “Tropicanna” through RAPD marker using 22 random primers. In the dendrogram, C. x generalis “President” and C. x generalis
“Grumpty”, both having deep red flowers, shared a common node with 65.4 % level of similarity. C. indica and C. indica “Tropicanna” were placed wide apart under two distant clades. Thus on the basis of RAPD, the findings of the study is at variance with that of Rajaseger et al. (1997 and 1999).

The inter-relationship obtained from RAPD analysis in Canna cultivars don’t fully conform the findings of Loh et al. (1999), who made AFLP analysis of two species and 7 cultivars of Caladium and inferred that closely related species can clearly be differentiated and that genetic difference between cultivars can also be established. At intra-specific level, two preliminary clusters could be identified in Caladium bicolor; one group contains the “fancy leaf” cultivars and the other group with “strap leaf” cultivars. There was also a single case of “fancy leaf” cultivar coming under “strap leaf” cultivar cluster. The plausible explanation offered was that in Caladium, difference in leaf shape might be due to allelic difference indicative of the fact that the dichotomy between fancy leaf and strap leaf may not be a very strong taxonomic character. Same inference may be drawn as to the flower colour in Canna and the reason for cultivars having same flower colour not coming together in the same cluster.

The genome relation revealed from ISSR marker system is different from RAPD analysis in several ways. In the dendrogram constructed using ISSR data, there were two clusters separated from each other at 41% level of similarity. While the first cluster contains the elemental species Canna indica and its hybrid C. indica “Tropicanna”, the second one holds all other cultivars of Canna x generalis complex except cv. Lucifer. Canna indica and C. indica “Tropicanna” shared a node at 47.6% similarity level. Similarly, Canna x generalis “President” - “Grumpty” and “King Humbert” - “Rosamond” had 58.4% and 67.6% similarity respectively.

The dendrogram constructed from the combined RAPD and ISSR data segregates the lone cultivar Canna X generalis “Lucifer” from rest of the varieties sharing a node only at 46.3% level of similarity. In ISSR analysis also, Canna x generalis “Lucifer” formed a group with Canna indica and C. indica “Tropicanna” and remained apart from rest of the cultivars of Canna x generalis complex. Similarly, Canna x generalis “Scarlet Beauty” was singled out in the second cluster from others at 51.8% similarity level. In subsequent sub-clusters, the elemental species C. indica and its hybrid C. indica “Tropicanna” got segregated from rest members of Canna x generalis at 53.4% level of similarity. Canna x generalis “President” made a cluster with “Grumpty” and “Rosamond” with “King Humbert” in the dendrogram with similarity values of 65% and 60.8% respectively.

The results obtained from RAPD, ISSR and the combination of these two markers revealed that it is not possible to clearly delimit elemental species and their hybrids/ forms and the horticultural taxa and their cultivars through molecular markers. Molecular data is also not helpful in segregating cultivars according to colours, height and parental origin but has some worth in deriving infra-specific genome relationship in certain cultivars and indicative of the fact that considerable genetic diversity do exist among cultivated species and cultivars of this popular group of garden plants.

In nature, Canna seems to have been evolved essentially by gene mutation and re-patterning of chromosomes. Man’s interference led to recombination emanating from inter-specific hybridization, somatic mutations and triploidy has speeded up the process of evolution (Khoshooh, 1979; Khoshooh and Mukherjee, 1970 b). As the distinction between the hybrid groups of Canna have been blurred by further inter-breeding, the cultivar names are not being used by many in horticultural treaties. The natural variability was also widening by spontaneously occurring mutations although the rate of such occurrence is very low. Canna being highly heterozygous where it takes longer duration between sexual generation, coupled with sexual sterility imposes strong restriction in its time consuming methods of hybridization and selection, hence, induced mutation may offer best tool to over come such restrictions.
The present study, though very preliminary in nature, brings out the facts and supports the earlier findings that sufficient genetic variability do exist within and among species, cultivars and hybrids of *Canna X generalis* and *Canna indica* which may be attributed to the history of origin, nature and genetic constitution of parent plants coupled with local climatic, edaphic and other environmental factors. The failure to delimit species and cultivars might be largely due to frequent inter-breeding involving one, two or more than two elemental and hybrid cultivars and forms.

In order to authentically establish the genetic variability and derive phylogeny among different species, species complexes, hybrids and cultivars, large number of species and cultivars with known origin and with larger representatives from each element should be analysed for molecular characterisation. Besides, other reliable and modern molecular techniques like AFLP, SSR etc. must be applied in addition to RAPD and ISSR for assessing the agro-biodiversity of Canna lilies and establishing phylogeny with relatively higher degree of accuracy.

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**References**


Sneath PHA and Sokal R (1973) Numerical Taxonomy. Freeman, San Francisco, California. 34.