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RESEARCH PAPER

Molecular profiling of 20 different accessions of *Canna* using RAPD and ISSR primers

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ABSTRACT

Canna, the solitary genus of the family Cannaceae, represents a group of ornamental plants. In the present study an attempt was made to access the genetic relationship among the 20 species and cultivars of *Canna* using RAPD and ISSR techniques. A total of 159 major scorable bands ranging from 220 to 1757 bp were generated from 18 RAPD primers showing 89.93% polymorphism. The lowest similarity was observed between *Canna edulis* and *Canna* x *generalis* Cv. "Trinacria Variegata" (52.9%), while the highest value was recorded between *Canna* x *generalis* Cv. "Dwarf Red" and *Canna* x *generalis* Cv. "Dwarf Orange" and between *Canna* x *generalis* Cv. "Dwarf Orange" and *Canna* x *generalis* Cv. "Froken" (94.1%). Ten ISSR primers resulted in 93 scorable bands showing 88.17% polymorphism among them. The band size ranged between 246 bp to 2017 bp. The highest correlation was found between *Canna edulis* and its green cultivar (96.4%), where as lowest was found between *Canna indica* and *Canna* x *generalis* Cv. "Orange Web" (55.9%). The dendrogram based on the combined data sets of both RAPD and ISSR showed considerable similarity with that obtained from individual RAPD and ISSR analysis, except for the position of *C. indica* Cv. Purpurea.

KEYWORDS: *Canna*; Molecular diversity, PEG, RAPD, ISSR.
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Introduction

The genus *Canna* comprises of about 51 species of flowering plants having flashy, brilliantly-coloured flowers and large tropical foliage. *Canna* species are native of South and North America and with the course of time they have been introduced in Asia and Europe and subsequently evolved into native varieties (Prince, 2010). The distribution of *Canna* species is most possibly the effect of human dispersal. The transportation of *Canna* from their native place might have been the reason for occurrence of beautiful ornamental plant in Europe, Asia and Africa (Maas-Van and Maas, 2008). Thousands of *Canna* hybrids are being used as cultivated garden ornamentals around the world. Some of the wild species of *Canna*, namely *C. glauca*, *C. indica*, *C. iridiflora*, *C. warscwiczii* and *C. flaccid* etc. are involved in producing natural as well as manmade hybrids. The above five species are popularly known as elemental species of *Canna*. The entire cultivated garden *Canna*s are included under two artificial hybrid species i.e. *Canna* x *orchiodes* L. H. Bailey and *Canna* x *generalis* L. H. Bailey (Hannay, 1936; Khoshoo and Mukherjee, 1970a). All the hybrid cultivars share some common features and bind themselves under the same horticultural species. Some morphological and physiological transformations might have occurred when they were shifted from wild to cultivated condition (Khoshoo and Mukherjee, 1970 b).

The taxonomy of Cannaceae is disputed as it relies on morphological features. Moreover, the distinguishing features between *Canna* varieties are poor as they lack proper molecular documentation. Till date not much work has been reported on the studies of genetic diversity of *Canna* cultivars in India and worldwide, except for Piyachomkwan *et al.*(2002); Patra *et al.* (2008) and Gupta *et al.* (2013) (Piyachomkwan *et al.* 2002; Patra *et al.* 2008; Gupta *et al.* 2013), who had done some experiments in a very limited way. Keeping this in mind, the present piece of work was designed to study the genetic diversity of *Canna* found in the Indian states of West Bengal and Odisha using RAPD (Random Amplified Polymorphic DNA) and ISSR (Inter Simple Sequence repeat) primers.

Materials and Methods

Collection of germplasm

In the present study, germplasm was collected from larger parts of West Bengal and Odisha. The collected samples were planted in the experimental garden of Molecular Genetics Laboratory, Department of Botany, North Bengal University, for further study after authentication by the plant taxonomists. List of different species and cultivars of *Canna* along with their morphological characters are given in Table 1.

Molecular diversity studies : PCR based molecular methods like, RAPD and ISSR markers were used for the genetic diversity study of different species and cultivars of *Canna*.

DNA extraction from leaf : Isolation of genomic DNA of *Canna* was carried out following Doyle and Doyle (1987) with minor modifications (Goyal and Sen, 2015).

Purification of Canna DNA

Major contaminants in crude DNA preparation are RNA, protein and polysaccharides and it is essential to remove them as these will hamper further downstream processing. Besides, it was found that *Canna* DNA always remain contaminated with starch when isolated through conventional procedure of Doyle and Doyle (1987). To get rid of this starch, we tried several methods, however, polyethyleneglycol (PEG, SIGMA) purification of *Canna* DNA was found to be the best which was done as per the following protocol.

One ml of DNA solution in TE was mixed with 400µl 30% weight/ volume (w/v) PEG solution (pH 7.4) and incubated for 14 hrs at 0ºC. The incubated mixture was centrifuged at 12,000 rpm (13,500Xg) for 30 min at 4ºC. The pellet obtained was resuspended in 500µl of 1X TE buffer. To this 0.1 volume of 3M sodium acetate (pH 5.5) and 2.5 volume of ice cold absolute ethanol was added for DNA precipitation.

The mixture of solution was incubated overnight at -20ºC and after incubation it was centrifuged at 13,000 rpm (16,000Xg) for 30 min at 4ºC. Transparent DNA pellet was obtained which was washed with 70% ethyl alcohol, air dried and finally dissolved in 500µl of 1XTE (pH 7.4) buffer. After PEG purification, the DNA was subjected to RNaseA treatment following the method of (Goyal and Sen, 2015).

RAPD and ISSR analysis

A total of 30 RAPD and 15 ISSR primers were screened for studying the genetic diversity among 20 different species and cultivars of *Canna*. RAPD and ISSR amplifications were performed using 25µl of PCR mixture containing 12.5µl PCR master Mix 2X (GeNei™), 1.25µl of primer (0.25 µM), 2µl of template DNA (25 ng/µl). The PCR reactions were performed on a Perkin-Elmer Thermocycler 2400.

Table 1. Morphological features of different species and cultivars of *Canna* collected from West Bengal and Odisha

Sample ID	Name of the Species/Cultivars	Phenotype of Species/Cultivars
C_1	*Canna edulis* Ker Gawler*	Plants tall; leaves broad, green with deep violet sheds; flowers small, red
C_2	*Canna edulis* Ker Gawler (green cultivar)*	Plant tall; leaves broad, green without any sheds; flowers small, reddish orange with yellow patches
C_3	*Canna indica* Linn.	Plant tall; leaves green; flowers small, red
C_4	*Canna* x *generalis* L.H. Bailey Cv. "Italia"	Plant tall; leaves green; flowers big, yellow with orange spots, sheds on the petals
C_5	*Canna* x *generalis* L.H. Bailey Cv. "Tropical Red"	Plant tall; leaves broad, green with red margin; flowers big, red
C_6	*Canna* x *generalis* L.H. Bailey Cv. "Orange web"	Plant tall; leaves broad, green with red margin; flowers big, orange
C_7	*Canna* x *generalis* L.H. Bailey Cv. "Austria"	Plant tall; leaves green; flowers big, yellow with red spots in the middle
C_8	*Canna* x *generalis* L.H. Bailey Cv. "President"	Plants short; leaves green with white boarder; medium sized, cherry red flowers
C_9	*Canna* x *generalis* L.H. Bailey Cv. "Pink silk"	Plants short; leaves green; big, light pink flowers
C_{10}	*Canna* x *generalis* L.H. Bailey Cv. "City of Portland"	Plants short; leaves green; flowers deep pink
C_{11}	*Canna indica* Linn. Cv. "Purpurea"	Plants tall; leaves green with violet sheds; flowers small, orange
C_{12}	*Canna* x *generalis* L.H. Bailey Cv. "Roi King Humbert"	Plants tall; leaves bronze; flowers dark red
C_{13}	*Canna* x *generalis* L.H. Bailey Cv. "Biercee"	Plants short; leaves green; flower creamish yellow with red throat
C_{14}	"*Canna* 21" (Crozy cultivar) [Unidentified]	Plants short; leaves green; flowers orange
C_{15}	*Canna* x *generalis* L.H. Bailey Cv. "Dwarf Yellow"#	Plants dwarf; leaves green; flowers yellow
C_{16}	*Canna* x *generalis* L.H. Bailey Cv. "Dwarf Red"#	Plants dwarf; leaves green with red margin; flowers dark red
C_{17}	*Canna* x *generalis* L.H. Bailey Cv. "Dwarf Orange"#	Plants dwarf; leaves green with red margin; flowers dark orange
C_{18}	*Canna* x *generalis* L.H. Bailey Cv. "Froken"#	Plants dwarf; leaves narrow; green; flowers yellow, spotted red
C_{19}	*Canna* x *generalis* L.H. Bailey Cv. "Jessica"	Plants short; leaves green; flowers pinkish orange
C_{20}	*Canna* x *generalis* L.H. Bailey Cv. "Trinacria Variegata"	Plants short; leaves variegated; flowers yellow

NB-* represents edible *Canna*, # represents dwarf cultivars, Sample ID "C1-C20" were used for further downstream processing

The condition of the thermal cycle for RAPD amplifications were 94ºC for 4 min, followed by 40 cycles of amplification with 1 min denaturation at 94ºC, 1 min annealing at 37ºC and 2 min primer extension at 72ºC and a final extension at 72ºC for 10 min. The condition of the thermal cycle for ISSR amplifications were 94ºC for 5 min, followed by 35 cycles of amplification with 45 seconds denaturation at 94ºC, 1 min annealing at 52ºC and 1 min primer extension at 72ºC and a final extension at 72ºC for 7 min. The PCR products were separated on 1.5% (w/v) agarose gel with two DNA markers such as λ DNA/*Eco*RI/*Hin*dIII double digest and a 100 base pair (bp) DNA ladder (GeNei™) and were photographed with Gel Documentation system (UVi).

Fingerprinting Data Analysis

Each polymorphic band was regarded as a binary character and was scored as 1 (presence) or 0 (absence) for each sample and assembled in a data matrix. To analyze the genetic relationship among the species and cultivars, a similarity matrix was calculated on the basis of the mathematical tools of Nei (Nei, 1972). Similarities were graphically expressed by using the UPGMA to generate dendrograms. The analysis was done by using Popgen32 software package (Yeh *et al*. 1997).

Correspondence analysis of right vectors from the binary data was performed on the basis of graphically summarized associations within the varieties. This analysis was performed through a batch file by using the software package NTSYSpc (version 2.0) (Rohlf, 1998).

Results and Discussion

DNA isolation and purification

The presence of various contaminants like polysaccharides, polyphenols and other secondary metabolites can interfere with the successful enzymatic reaction with DNA. Specifically, the polysaccharides in DNA inhibit Taq DNA polymerase activity (Fang *et al*. 1992). The extraction of pure DNA is very essential for further downstream processes like PCR amplification. In case of *Canna*, whitish viscous mass of polysaccharide co-precipitated with DNA during the early stage of DNA extraction. Similar problem was also reported in *Entelea arborescens*, where isolated DNA was contaminated with highly viscous mass of polysaccharides (Shepherd and McLay, 2011). So it was obvious that addition of CTAB in the DNA extraction buffer failed to eliminate the starch compound completely from the DNA. So in *Canna*, the DNA isolated through CTAB method was further extracted once with phenol: chloroform: isoamyl alcohol, followed by purification in polyethyleneglycol (PEG) for removal of polysaccharides. The RNAase A enzyme was found to be effective in eliminating RNA. After the above purification steps, the DNA pellet were completely soluble in TE and was in pure form which was suitable for further downstream processing.

RAPD analysis

In the present study, genetic diversity has been studied using 30 RAPD primers among different species and cultivars of *Canna* found in West Bengal and Odisha. Out of 30 RAPD primers, 18 yielded reproducible RAPD bands in different species/cultivars. A total of 159 major scorable bands ranging from 220 base pair (bp) to 1757 bp were generated, out of which only 143 fragments were polymorphic showing a polymorphic percentage of 89.93% (Table 2). The number of polymorphic bands generated by each decamer primers ranged in between 1 (OPA20 and OPN13) and 13 (OPA01 and OPA17). The RAPD profile of the 20 accessions of *Canna* generated using primers OPA03 and OPH04 are depicted in figure 1. Some primers like OPA03, OPA18, OPB01, OPG19, OPH04 and OPN05 showed 100% polymorphism among the cultivars owing to their self incompatibility. This makes them highly heterogeneous and consequently shows broad genetic variation among them. All the other primers showed more than 75% polymorphism except for OPA20 and OPN13, where polymorphism was 20% and 33% respectively. Similarity coefficient of 20 accessions ranged from 0.529-0.941. The lowest similarity was observed between C_1 (*Canna edulis* Ker Gawler) and C_{20} (*Canna* x *generalis* Cv. "Trinacria Variegata"), while the highest value was recorded between C_{16} (*Canna* x *generalis* Cv. "Dwarf Red") and C_{17} (*Canna* x *generalis* Cv. "Dwarf Orange") and between C_{17} (*Canna* x *generalis* Cv. "Dwarf Orange") and C_{18} (*Canna* x *generalis* Cv. "Froken"). The dendrogram constructed on the basis of the data obtained from RAPD analysis using Popgen32 is represented in Figure 2. Among the 20 different accessions, we had 2 different elemental species having two cultivars each and 16 hybrid cultivars. In the dendrogram, both the cultivars of *Canna edulis* came together. However, two varieties of *Canna indica* were separated out. This may be due to geographical barrier between the two cultivars and thus, they evolved independently. Even morphologically also, these two cultivars were quite different. *Canna indica* Cv. "Purpurea" have purpulish green leaves with orange flowers, whereas the other variety had green leaves with red flowers. Regarding the hybrid cultivars, all of them flocked together in a big cluster except *Canna* x *generalis* Cv. "City of Portland". In this big cluster, we could recover all four dwarf varieties, namely *Canna* x *generalis* Cv. "Dwarf Yellow", *Canna* x *generalis* Cv. "Dwarf Red", *Canna* x *generalis* Cv. "Dwarf Orange" and *Canna* x *generalis* Cv.

"Froken" which grouped together. The dwarf Red and dwarf Orange cultivars showing a node at 94.1% proximity level, were found to be the closest among all the hybrid plants. Thus, the dendrogram was broadly divided into two clusters, the smaller one contained two different elemental species and the larger cluster contained all the available hybrid cultivars of *Canna*. On the basis of similarity indices, first sub-cluster of dendrogram showed two cultivars of same species i.e. *Canna edulis* and *Canna edulis* green cultivar sharing a node at 89%. Similar result was reported by Piyachomkwan *et al.* (2002), where high level of genetic similarity was found between indigenous Thai cultivars of *Canna edulis*. Further, *Canna indica* was placed in the first cluster with *Canna edulis and Canna edulis* green cultivar, sharing the similarity level of 67.2% and 73.1% respectively. All the 17 ornamental cultivars were hybrid plants and grouped together to form the larger cluster. It was hypothesized that the group of garden plants were clustered together to form a somaclone complex, which might have originated from somatic mutation of single cultivar at different times and different geographical locations (Ude *et al.* 2003). Though morphological diversity was seen among the members of somaclone complex, they may share some common characters which bound them under single horticultural species i.e. *Canna generalis* Bailey and distinguished them from the elemental species which were responsible for their origin (Khoshoo and Mukherjee, 1970).

The cultivars of the horticultural complex were grouped in 2 different sub-clusters on the basis of various morphological and physicochemical characters namely plant height, flower colour, flower size, blooming period etc. Among the hybrids, *Canna* x *generalis* Cv. "Roi King Humbert" was grouped together with *Canna* x *generalis* Cv. "Austria", "Orange Web", "Tropical Red", "Italia" and "Trinacria Variegata" to form the first sub-cluster.

Table 2. Total number and size of amplified bands, number of polymorphic and monomorphic bands and percentage of polymorphism generated by the RAPD primers

Primer	Seq. (5'-3')	Band No.	MB	PB	Pol%	Band size
OPA01	CAGGCCCTTC	14	1	13	92.85%	400-1700
OPA02	TGCCGAGCTG	10	1	9	90%	348-1757
OPA03	AGTCAGCCAC	12	0	12	100%	305-1650
OPA04	AATCGGGCTG	6	1	5	83.33%	220-1200
*OPA05	ATTTTGCTTG	–	–	–	–	–
*OPA06	GGTCCCTGAC	–	–	–	–	–
OPA07	GAAACGGGTG	11	1	10	90.9%	432-1600
*OPA08	GTGACGTAGG	–	–	–	–	–
*OPA09	GGGTAACGCC	–	–	–	–	–
OPA10	GTGATCGCAG	7	1	6	75%	550-1600
OPA11	CAATCGCCGT	11	1	10	90.9%	250-1170
OPA17	GACCGCTTGT	14	1	13	92.85%	230-1214
OPA18	AGGTGACCGT	7	0	7	100%	570-1385
*OPA19	CAAACGTCGG	–	–	–	–	–
OPA20	GTTGCGATCC	5	4	1	20%	400-1142
OPB01	GTTTCGCTCC	10	0	10	100%	533-1463
*OPB02	TGATCCCTGG	–	–	–	–	–
*OPB03	CATCCCCCTG	–	–	–	–	–
*OPB04	GGACTGGAGT	–	–	–	–	–
*OPB05	TGCGCCCTTC	–	–	–	–	–
*OPB06	TGCTCTGCCC	–	–	–	–	–
*OPB07	GGTGACGCAG	–	–	–	–	–
*OPB08	GTCCACACGG	–	–	–	–	–
OPF09	CCAAGCTTCC	6	1	5	83.33%	359-1099
OPG19	GTCAGGGCAA	8	0	8	100%	461-1422
OPH04	GGAAGTCGCC	9	0	9	100%	335-1134
OPN04	GACCGACCCA	10	1	9	90%	320-1115
OPN05	ACTGAACGCC	9	0	9	100%	490-1612
OPN13	AGCGTCACTC	3	2	1	33.33%	614-986
OPN19	GTCCGTACTG	7	1	6	85.71%	410-1205
Total		**159**	**16**	**143**		

*Not amplified; MB=Monomorphic bands; PB=Polymorphic bands; Pol%=%age of polymorphism.

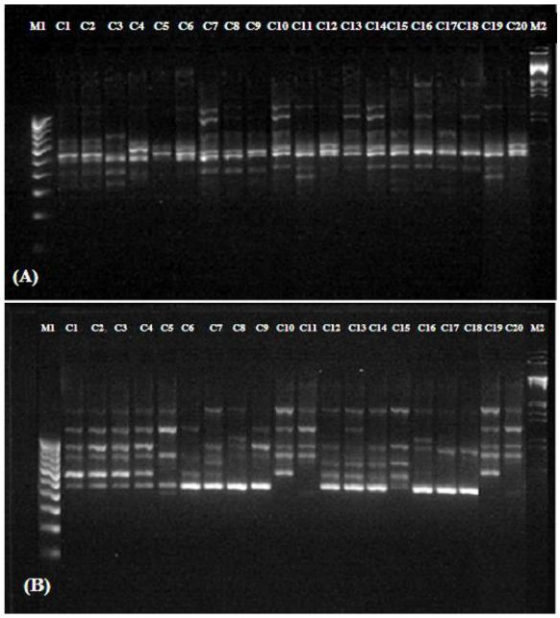
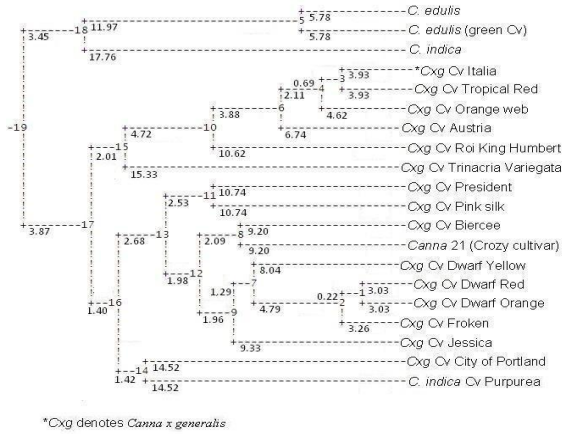


Figure 1. A representative of RAPD profile of 20 accessions of *Canna* amplified with (A) OPA03 primer and (B) OPH04 primer. Lane M1: 100 bp molecular marker; Lane C1- C2O: different accessions of *Canna* under study (Please refer table 1 for the name of the species and cultivars); Lane M2: λ DNA/*Eco*RI/*Hind*III double digest DNA ladder.



*Cxg denotes *Canna x generalis*

Figure 2. Dendrogram based on Nei's (1972) UPGMA method, modified from NEIGHBOR procedure of PHYLIP Version 3.5 (RAPD markers), illustrating the genetic relationship among 20 accessions of *Canna*. Numbers at nodes indicate the bootstrap values and at the bottom of the lines are root distances.

The above 6 cultivars were tall plants having large and bright flowers. On the basis of their phenotypic characters like plant height and flower pattern, they resemble each other and grouped together to form the sub-cluster. From the above result it could be assumed that variegation in the leaf structure of Cv. "Trinacria Variegata" may not be considered as a distinct phenotypic character for segregation of a cultivar from a particular group (Percy-Lancaster, 1927). The second sub-cluster had 2 groups. It was observed that 9 garden cultivars such as *Canna* x *generalis* Cv. "President", "Pink silk", "Biercee", "*Canna* 21" (an unidentified Crozy cultivar), "Jessica" and 4 dwarf varieties were placed together to form a bigger group. The above plants were assembled together because of their short height with narrow and green foliage. Further *Canna* x *generalis* Cv. "City of Portland" and *Canna indica* Cv. "Purpurea" were clustered together to form the smallest group as they had medium height, same blooming period and had similar geographical locations. The above findings were in accordance with Patra *et al.* (2008), where morphologic characters were focused in discussing the molecular variability of different cultivars of *Canna*. In the present study, though morphological character was considered for analyzing the genetic variability, it was observed that the big pink flowered cultivars like *Canna* x *generalis* Cv. "Pink silk", "Jessica" and "City of Portland" were distantly placed in the dendrogram. This result was in close correspondence with the phylogenetic evaluation of Patra *et al.* (Patra *et al.* 2008), where two *Canna* cultivars having deep red flowers were placed wide apart under two different clades. Thus it could be inferred that some allelic characters may be responsible for placing different pink flowered plants at a distance from each other (Loh *et al.* 1999).

The present study demonstrates that RAPD offers a suitable means for detecting genetic diversity in *Canna*.

ISSR analysis

Fifteen ISSR primers were initially screened to generate polymorphic bands in studied *Canna* samples, out of which only 10 primers were able to produce distinct, scorable bands (Table 3) and were selected for further study. A total of 93 bands were produced having band size ranging between 246 bp to 2017 bp. Highest number of bands (15 bands) were generated by UBC873, whereas UBC841 produced the lowest number of bands (6 bands). The frequency of polymorphism was found to be 88.17%. Two primers (UBC815 and UBC824) showed 100% polymorphism among the cultivars, where as all other primers produced more than 75% polymorphism except for UBC841 (40%). A representative of ISSR profile of the 20 accessions of *Canna* generated with primers UBC873 and UBC815 is depicted in Figure 3. Nei's genetic similarity between each pair of species ranged between 0.559 - 0.964. The highest correlation was found between *Canna edulis* and it's green cultivar, where as lowest was found

between *Canna indica* and *Canna* x *generalis* Cv. "Orange Web". The dendrogram (Fig. 4) prepared out of ISSR analysis results indicated that *Canna indica* is the out-group in the tree and therefore, it became considered as the root. *Canna indica* Cv. "Purpurea" is placed closest to the root. Both the varieties of *Canna indica* were elemental species and known to be involved in the creation of hybrid cultivars (Khoshoo and Mukherjee, 1970; Patra *et al*. 2008). This result was in correspondence with the phylogenetic analysis study of *Canna* by Patra and his coworkers in 2008. They observed *Canna indica* and it's hybrid *Canna indica* "Tropicanna" segregated out from the rest of the cultivars of *Canna* x *generalis* complex. Then there were three distinct clades. One was with two elemental varieties, *Canna edulis* and *Canna edulis* green cultivar. Being the cultivars of the same species, they had a cluster sharing node at 96.4%, showing the highest similarity coefficient in the dendrogram. High level of similarity between these two cultivars was reported by Piyachomkwan and his coworkers (Piyachomkwan *et al*. 2002). The next clade consisted of 7 hybrid cultivated varieties of *Canna* x *generalis* Cv. "Italia", "Austria", "Tropical Red", "Orange Web", "Roi King Humbert", "Jessica", "Trinacria Variegata". These 7 hybrids of *Canna* x *generalis* were assembled together as that of RAPD, but the only difference was that *Canna* x *generalis* Cv. "Jessica" was included within the group. Whereas, the other clade consisted of 9 hybrid cultivated varieties namely, *Canna* x *generalis* Cv. "President", "Pink silk", "City of Portland", "Biercee", "*Canna* 21" and all the 4 dwarf varieties. These 9 ornamental cultivars clustered together as a somaclone complex as described in case of RAPD primers (Ude *et al*. 2003). The dwarf varieties, which clustered together in RAPD tree, also clustered here except the *Canna* x *generalis* Cv. "Dwarf Yellow", which clustered with non dwarf varieties like Cv. "Biercee" and "*Canna* 21". However, in RAPD tree also, we found that out of 4 dwarf varieties, dwarf Yellow cultivar was relatively distant from rest of the three. This showed that the Yellow dwarf variety perhaps evolved differently than that of the other dwarf varieties. Thus, it can be inferred from the ISSR marker study that along with morphological features, phylogenetic origin of plants could be considered for the correct taxonomic differentiation.

Combined RAPD and ISSR based analysis

Both RAPD and ISSR markers were combined to construct the phylogenetic tree of 20 different accessions of *Canna*. The similarity coefficients based on 159 RAPD and 93 ISSR loci ranged in between 0.591 to 0.936. Unlike RAPD and ISSR data, the highest correlation was found in between *Canna* x *generalis* Cv. "Tropical Red" and *Canna* x *generalis* Cv. "Orange Web" (0.936) while the lowest was found between *Canna* x *generalis* Cv. "Dwarf Red" and *Canna edulis* (0.591). Cluster analysis performed from the combined data of both RAPD and ISSR markers generated a dendrogram which is illustrated in Figure 5.

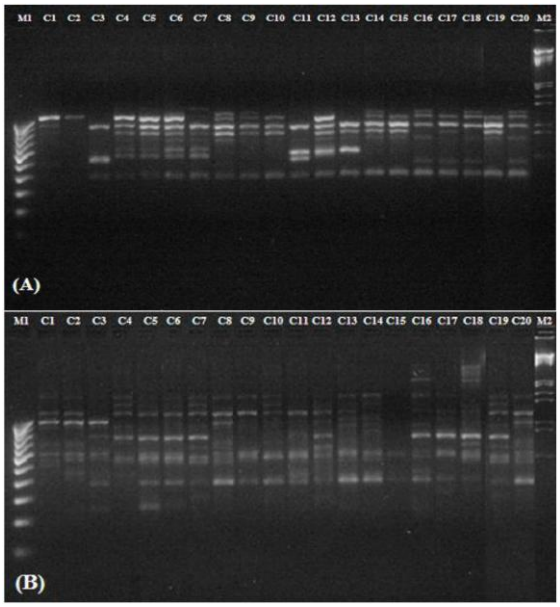


Figure 3. A representative of ISSR profile of 20 accessions of *Canna* generated by (A) UBC815 primer and (B) UBC873 primer. Lane M1: 100 bp molecular marker; Lane C1- C2O: different accessions of *Canna* under study; Lane M2: λ DNA/*Eco*RI/*Hind*III double digest DNA ladder.

The principal Coordinate Analysis which is based on the similarity coefficients or variance-covariance among the traits validated the dendrogram (Akond *et al*. 2007). The correspondence analysis (Fig. 6) was in accordance with the cluster analysis results. In the 2-dimentiopnal plot of the Principal Coordinate Analysis, there are 4 quadrates on the basis of the values of X and Y axis. Both X and Y axis of the upper right quadrate is positive, where as lower right quadrate is X positive and Y negative. Similarly, both X and Y axis of the lower left quadrate is negative, where as upper left quadrate is X negative and Y positive. This plot shows that the elementary species i.e. *C. edulis* and it's green cultivar and *C. indica* fell in positive X and Y axis, where as almost all the hybrid cultivars dropped in negative X and Y axis. Very few cultivars clustered in other two quadrates. All the three dendrograms (RAPD, ISSR and combined) were similar to each other in many ways, such as grouping of garden cultivars as a somaclone complex, separation of elemental species from the hybrid cultivars

Table 3. Total number and size of amplified bands, number of polymorphic and monomorphic bands and percentage of polymorphism generated by the ISSR primers

Primer ID	Sequence (5'-3')	Total bands amplified	Monomorphic bands	Polymorphic bands	Percentage of polymorphism	Band size (bp)
*UBC807	(AG)8T	—	—	—	—	—
*UBC808	(AG)8C	—	—	—	—	—
UBC810	(GA)8T	13	1	12	92.30%	447-1517
*UBC811	(GA)8C	—	—	—	—	—
UBC813	(CT)8T	9	1	8	88.88%	310-1185
UBC815	(CT)8G	10	0	10	100%	348-1152
UBC818	(CA)8G	14	1	13	92.85%	280-1436
UBC822	(TC)8A	8	2	6	75%	574-1860
UBC824	(TC)8G	6	0	6	100%	481-1520
UBC825	(AC)8T	5	1	4	80%	351-1175
*UBC834	(AG)8YT	—	—	—	—	—
*UBC836	(AG)8YA	—	—	—	—	—
UBC841	(GA)8YC	5	3	2	40%	246-1225
UBC856	(AC)8YA	8	1	7	87.5%	265-866
UBC873	(GACA)4	15	1	14	93.33%	419-2017
Total		**93**	**11**	**82**		

*Not amplified

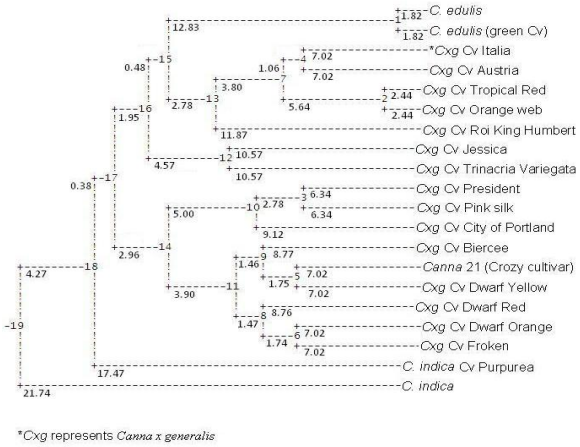


Figure 4. Dendrogram based on Nei's (1972) UPGMA method, modified from NEIGHBOR procedure of PHYLIP Version 3.5 (ISSR markers), illustrating the genetic relationship among 20 accessions of *Canna*. Numbers at nodes indicate the bootstrap values and at the bottom of the lines are root distances.

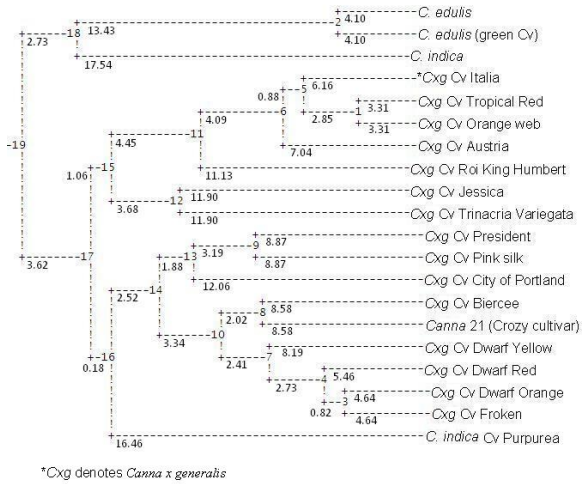


Figure 5. Dendrogram based on Nei's (1972) UPGMA method, modified from NEIGHBOR procedure of PHYLIP Version 3.5 (combined RAPD and ISSR markers), illustrating the genetic relationship among 20 accessions of *Canna*. Numbers at nodes indicate the bootstrap values and at the bottom of the lines are root distances.

and the closeness of four dwarf cultivars etc. The only difference was that *Canna indica* Cv. "Purpurea" was separated from *Canna indica* in the RAPD and combined tree. However, the RAPD based tree was found to be more similar with combined tree than that of ISSR tree. The results obtained from RAPD, ISSR and the combination of these two markers revealed that it is possible to separate the elemental species from the horticultural taxa and their cultivars through molecular markers. The phenotypic characters along with phylogenetic origin of plants should be preferred as suitable parameters to prevail over taxonomic complexities.

The cultivars cannot be segregated from the somaclone complex just based on the colour, height and parental origin, but some significant infra-specific genome relationship might also be considered. This indicates that considerable genetic diversity do exists among the cultivars of popular garden plants. The present study supports the earlier findings that sufficient genetic variability exists within and among the species, cultivars and hybrids of the horticultural species, attributing to the origin, nature and genetic constitution of the parent plant coupled with

climatic, edaphic and other environmental factors. Thus, overall DNA fingerprinting study of *Canna* cultivars have generated lots of new markers for identification of *Canna* and can prove to be effective and promising in assessing genetic variations among the ornamental cultivars collected from various places of West Bengal and Odisha.

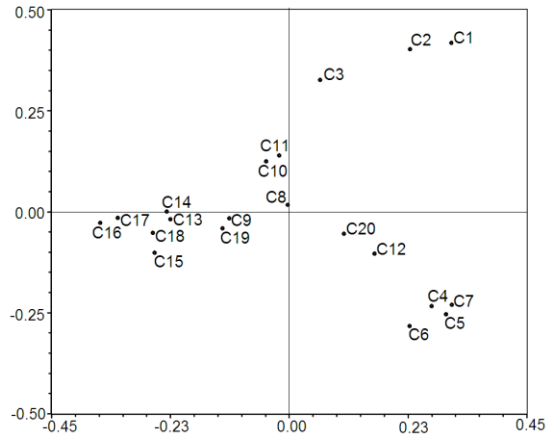


Figure 6. Principal Coordinate Analysis (2-dimensional plot) of 20 accessions of *Canna* based on combined RAPD and ISSR analysis data. C1- C2O: different accessions of *Canna* under study.

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