RESEARCH ARTICLE

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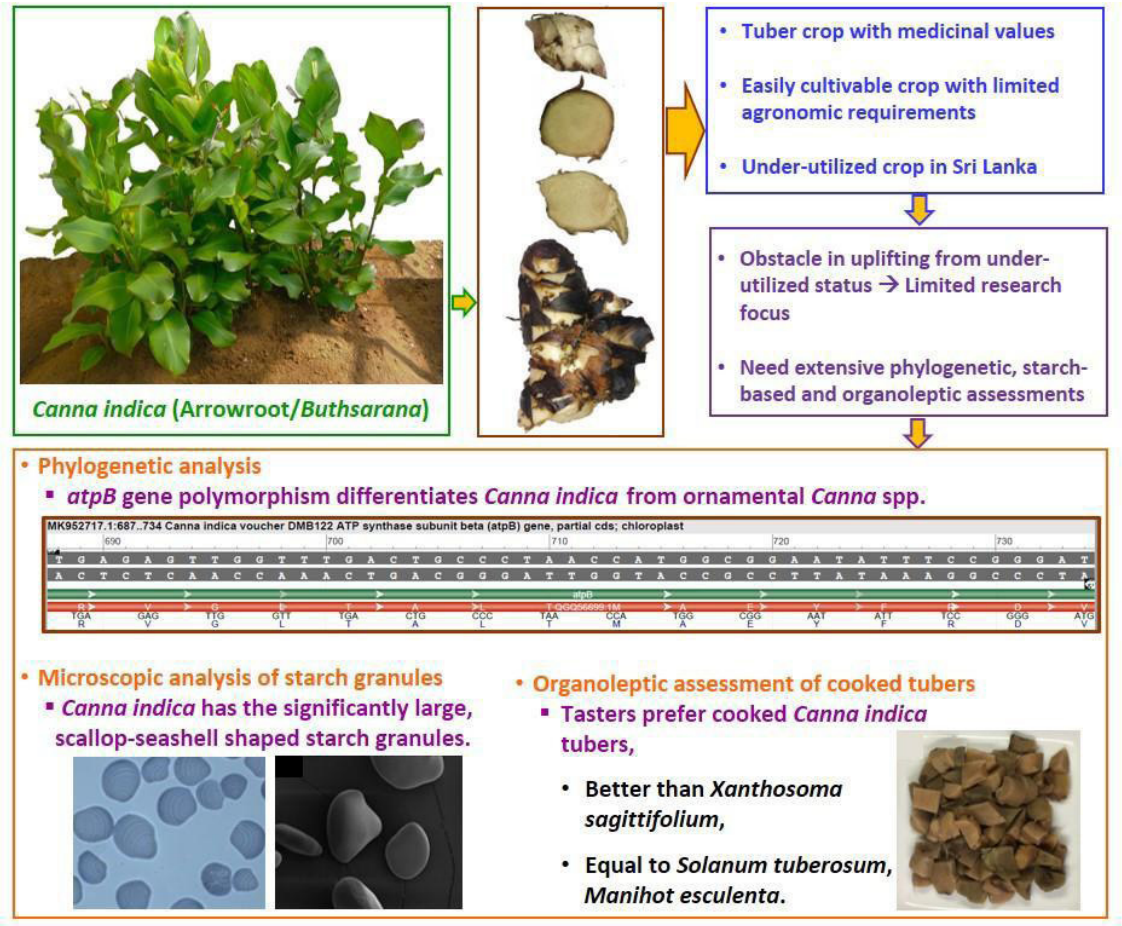

Highlights

- The sequence polymorphism of the *atpB* gene differentiates *Canna indica* (Arrowroot/*Buthsarana*) from ornamental *Canna* spp.
- *Canna indica* has the significantly large starch granules compared to those of *Xanthosoma sagittifolium*, *Manihot esculenta*, *Solanum tuberosum*, and *Ipomoea batatas.*
- *Canna indica* has unique scallop-seashell shaped starch granules.
- The cooked *Canna indica* tubers are accepted, better than those of *Xanthosoma sagittifolium*, and rated equally to *Solanum tuberosum*, and *Manihot esculenta.*

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Analyses of phylogenetics, starch granule morphology and consumer preference of *Canna indica* L. grown in Sri Lanka

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Abstract: *Canna indica* is a tuber crop which has many medicinal values. In Sri Lanka, *C. indica* tubers are consumed in rural areas and mainly available in street-markets of Nuwara-Eliya and Kandy Districts. In the present study, we assessed the phylogenetics of *C. indica*, starch granule morphology and consumer preference of *C. indica* tubers in comparison to the popular tuber crops. The phylogenetic analysis was conducted based on the sequence polymorphism at *rbc*L, *atp*B gene, *trn*L-*trn*F and *trn*H-*psb*A marker-loci with respect to the ornamental *Canna* spp. in Sri Lanka and the previously published sequences of *Canna* spp. The starch granules were isolated and observed under optical and scanning electron microscopes. The diameter and the surface area of the starch granules were measured under the optical microscope and subjected to analysis of variance. As *C. indica* tubers are consumed as boiled tuber pieces in Sri Lanka, the consumer preference analysis was conducted using the boiled tuber pieces *C. indica*, *Xanthosoma sagittifolium*, *Manihot* esculenta, *Solanum tuberosum*, and *Ipomoea batatas*. The phylogenetic tree based on *rbc*L marker revealed that *C. indica* in Sri Lanka is slightly divergent from the other *Canna* spp. Only the polymorphism of the *atp*B gene can be used to differentiate *C. indica* from the ornamental *Canna* sp. in Sri Lanka. The morphological analysis of starch granules revealed that *C. indica* has the biggest scallop-seashell shaped starch granules compared to other tuber species. The boiled *C. indica* tubers were accepted better than that of *X. sagittifolium*, rated equally to the tubers of *S. tuberosum* and *M. esculenta*, and rated less than *I. batatas*. The hardy and fibrous nature of *C. indica* tubers must be the major limiting factors for achieving the highest consumer preference highlighting the need of breeding for better texture in tubers.

Keywords: *Devkali*, Indian-shot, Large starch granules in plants, Ornamental *Canna* spp., Underutilized tuber crops.

INTRODUCTION

Canna indica of family Cannaceae is considered as one of the traditionally used tuber crops in the world (Prince, 2010). The vernacular names of *C. indica* are Indian-Shot in English, Arrowroot in Africa, *Achira* in Latin America, *Devkali* in India, *Buthsarana* in Sinhala, and Ampurut Alai in Tamil (Bachheti *et al*., 2013). It is believed that *C. indica* was originated in Latin America (Andrade-Mahecha *et al*., 2012) and later naturalized in many countries including Sri Lanka (Olango *et al*., 2013; Gunarathna *et al*., 2016). The rhizome is the edible part of the plant and all parts of the plant contain medicinal values including antioxidant property (Mahesh *et al*., 2014; Joshi *et al*., 2009a), HIV reverse transcription inhibitory activity (Woradulayapinij *et al*., 2005), anti-diarrheal, hemostatic, hepatoprotective, antibacterial and anti-worming effects (Rahmatullah *et al*., 2010; Josephine *et al*., 2013; Lin *et al*., 2011; Joshi *et al*., 2009b; Anisuzzaman *et al*., 2007; Indrayan *et al*., 2011; Thepouyporn *et al*., 2012; Abdullah *et al*., 2012; Nirmal *et al*., 2007).

The tubers of *C. indica* are a common commodity in ayurvedic herbal medical preparations (Kankanamalage *et al*., 2014). Although *C. indica* possesses significant medicinal and food values, it is currently an underutilized tuber crop in Sri Lanka (Malkanthi, 2017). The tubers of *C. indica* rarely appear in the country-wide market. The younger generation who generally appreciate fast food and limited array of choices, even do not know that *C. indica* tuber is a human food. However, *C. indica* is fast growing (Zhang *et al*., 2008) with an excellent ornamental value (Zhang *et al*., 2008) and recently being identified as one of the best species for the phytoremediation of the polluted water released from reverse osmosis process of the water purification units in Sri Lanka (Gunarathna *et al*., 2016). It is believed that *C. indica* contains the largest starch granules ever known (Hermann *et al*., 1997) thus, can be used as a candidate species to broaden our knowledge on starch science and technology.

In Sri Lanka, *C. indica* is grown in lowland plains (30 – 200 m above sea level) of Sri Lanka without much attention. However, *C. indica* plants thrive well in the central highlands of Sri Lanka (500 m above mean sea level, 2000 mm annual rainfall and 23-26 ^{0}C of mean temperature) and grow as large dense bushes where suckers keep coming from the mother plants. The tubers can be harvested for human

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consumption after six months of planting. There are slight variations in leaf size, leaf margin and plant height exist among the *C. indica* populations found in different habitats. However, when suckers from one location is planted in a different habitat, these variations get changed based on the soil fertility and moisture content. However, the genetic diversity or the exact species delimits of the *C. indica* have not been studied in Sri Lanka. In addition to the edible *C. indica*, there are ornamental *Canna* plants which can produce attractive flowers and foliage grown in many places of the country. The species differences between edible *C. indica* (i.e. Arrowroot) and the ornamental *Canna* spp. have not been studied to date. The extensive multidisciplinary research attempts are needed to uplift *C. indica* from its current underutilized status. Also, the taste and other sensory attributes of *C. indica* in comparison to the popular tuber crops must be studied. Therefore, the present study was conducted to assess the species delimits and phylogenetic relationships, starch granule morphology, and consumer preference of *C. indica* in Sri Lanka.

MATERIALS AND METHODS

DNA extraction, PCR and DNA sequencing

The DNA was extracted from the immature leaves of *C. indica*, plants collected from Kundasale, Matale, Nuwara-Eliya, Peradeniya (7°16'42.2"N 80°40'33.7"E, 7°27'29.7"N 80°36'31.3"E, 6°52'53.7"N 80°48'55.5"E, 7°16'01.1"N 80°36'23.2"E) and ornamental *Canna* spp. plants in Kandy, (7°15'26.8"N 80°35'08.9"E) using modified cetyltrimethylammonium bromide (CTAB) method (Porebski *et al*., 1997). The extracted DNA samples were stored at -20 °C. The PCR was performed in a Thermal Cycler (TP600: Takara, Otsu Shiga, Japan) using four DNA barcoding markers given in Table 1. Each PCR was performed in a 30 µl reaction mixture with 2× Go Taq Green Master Mix (15 µl), 10µM forward and reverse primers (1 µl each) and 10 µM spermidine (7 µl). The PCR products were resolved in 2.5% agarose gel electrophoresis (Sambrook and Russell, 2001). The PCR amplicons of the four DNA markers were purified using QIAquick® PCR purification kit (Catalog No.: 28104, Qiagen, Hilden, Germany) and sequenced using ABI 3500 Automated Sequencer (Catalog No.: 622-0010, Applied Biosystem). All the sequences generated were submitted to the Genbank under the accession numbers: MK952702-MK952733.

Phylogenetic analysis

The initial and end noises of the sequencing data obtained from four DNA markers (Table 1) were observed independently using MEGA 7 (Kumar *et al*., 2016). Then the identities of the trimmed sequences were confirmed independently by subjecting to a search in Basic Local Alignment Search Tool (BLAST). We created separate alignments for each marker in MEGA 7. In order to relax the family Cannaceae topology, we did not use phylogenetically distant outgroup, or a too closely related one. Thus, we used two species from family Commelinaceae as outgroups which can also be found in the Commelinids clade where family Cannaceae is located. To compare the *Canna* spp. Sri Lanka, the sequences generated were aligned for the marker, *rbc*L, with the dataset generated in Prince, (2010) (Table 2). A model selection analysis in Jmodel test (Posada, 2008) was implemented to infer the best nucleotide substitution process of the dataset. A phylogeny was constructed in Maximum Likelihood framework in RAxML (Stamatakis, 2006). We implemented rapid+boostrap algorithm with 1000 iterations and GTRGAMMA as the nucleotide substitution model. The analysis was implemented in the CIPRES supercomputer (Miller *et al*., 2010). We also carried out a tree search in the Bayesian framework in MrBays (Huelsenbeck and Ronquist, 2001) in CIPRES science gateway. Two hot and cold chains of Markov Chain Monte Carlo (MCMC) running for 30 million generations were used to probe trees in the tree-space. The 10% of the initial trees were removed as burn-in, and the rest of the probed trees were used to draw the final 50% majority rule consensus tree. The Effective Sample Size (ESS) of all the priors used were checked in TRACER v1. 4 (Rambaut and Drummond, 2007). To check the intra species variability of the Sri Lankan *Canna* spp., UPGMA trees were constructed for the markers, using uncorrected pairwise genetic distance matrices. The gaps (INDELS) were considered as pairwise deletions and considered uniformed rates across all the states. The trees were constructed in MEGA7. Finally, all the drawn trees were modified using FigTree v1.4.3 (Rambaut, 2014).

Table 1: DNA barcoding markers used in the present study.

DNA marker	Primer sequence	Annealing temperature (°C)	Reference
*trn*H-*psb*A	F: CGCGCATGGTGGATTCACAATCC	64	Tate and Simpson, (2003)
	R: GTTATGCATGAACGTAATGCTC		Sang *et al*., (1997)
*rbc*L	F: ATGTCACCACAAACAGAGACTAAAGC	55	Levin *et al*., (2003)
	R: GTAAAATCAAGTCCACCRCG		Kress *et al*., (2009)
*trn*L-*trn*F	F: CGA AAT CGG TAG ACG CTA CG	50	Taberlet *et al*., (1991)
	R: ATT TGA ACT GGT GAC ACG AG		
*atp*B *gene*	F: TATGAGAATCAATCCTACTACTTCT	55	Hoot *et al*., (1995)
	R: TCAGTACACAAAGATTTAAGGTCAT		

Table 2: Metadata of the sequences used in the phylogenetic analysis.

Species	Voucher No.	GenBank accession numbers			
		trnH-psbA	***atpB gene***	***rbcL***	***trnL-trnF***
C. indica (Kundasale: arrowroot)	DMB115	MK952726	MK952710	MK952702	MK952718
C. indica (Matale: arrowroot)	DMB116	MK952727	MK952711	MK952703	MK952719
C. indica (Ambewela: arrowroot)	DMB117	MK952728	MK952712	MK952704	MK952720
C. indica (Nuwara-Eliya: arrowroot)	DMB118	MK952729	MK952713	MK952705	MK952721
C. indica (Peradeniya: arrowroot)	DMB119	MK952730	MK952714	MK952706	MK952722
C. indica (Kandy: ornamental red)	DMB120	MK952731	MK952715	MK952707	MK952723
C. indica (Kandy: ornamental yellow)	DMB121	MK952732	MK952716	MK952708	MK952724
C. indica (Kandy:ornamental orange)	DMB122	MK952733	MK952717	MK952709	MK952725
C. flaccida	Siebert_1403			FJ861136	
C. glauca	Prince_1995 239			AF378774	
C. indica	Kress_89 2849			AF378763	
C. indica	Godfrey_60501			FJ861135	
C. indica	Duke_88 124			FJ861130	
C. indica	Duke_66 314			FJ861131	
C. indica	Prince_1995 210			FJ861132	
C. iridiflora	Plowman Davis_4753			FJ861134	
C. jaegeriana	Kress_89 2884			FJ861133	
C. paniculata	Plowman Kennedy 5700			AY656132	
C. tuerckheimii	Kress_89 2853			AF378764	
C. tuerckheimii	Duke_85 034			FJ861129	
Costus pulverulentus	Duke_GH_86 043			AY656108	
Dimerocostus strobilaceus	Kress_94 3601			AF243838	
Tapeinochilos ananassae	Kress_90 2984			AF243840	
Heliconia irrasa	Kress_76 519			AF378778	
Heliconia rostrata	Duke_GH_81 030			AF378767	
Orchidantha fimbriata	Kress_87 2159			AF243841	
Orchidantha siamensis	Kress_94 3718			AF378771	
Haumania sp	DJ_Harris_6672			AY656119	
Sarcophrynium brachystachys	Kress_01 7007			AY656126	
Ensete ventricosum	Kress_96 5372			AF243843	
Musa ornata	Duke_GH_88 110			AF378779	
Musella lasiocarpa	Kress_94 3709			AF243844	
Phenakospermum guyannense	Kress_86 2099D			AF243845	
Ravenala madagascariensis	Kress_92 3504			FJ861128	
Strelitzia nicolai	Duke_GH_78 044D			AF243846	
Globba curtissii	Kress_99 6247			AF243847	
Siphonochilus kirkii	Kress_94 3692			FJ861127	
Anigozanthos sp	Prince_2003 499			FJ861123	
Cartonema philydroides	-			FJ861121	
Hanguana sp	Kress_99 6325			FJ861125	
Helmholtzia glaberrima	Prince_2003 007			FJ861124	
Palisota ambigua	Faden_86/55			FJ861122	
Dyckia marnier-lapostollei	T._World_97686			FJ861120	

Assessment of the starch granules

The starch granule morphology of *C. indica* was assessed in comparison to the starch granules of well-known tuber crops in Sri Lanka; *Xanthosoma sagittifolium*, *Manihot esculenta*, *Solanum tuberosum*, *Ipomoea batatas* purple-tuber, and *I. batatas* yellow-tuber. The healthy tubers at the right maturity stage for harvesting were collected from all six species.

Isolation of starch

The starch was isolated from each species using the modified protocols explained in Alves *et al*., (1999) and De Pater *et al*., (2006). The tubers were thoroughly washed, peeled and chopped into small pieces. The chopped pieces were homogenized using a solution containing 0.075% sodium bisulfate to avoid the browning. The homogenate-slurry was then filtered to eliminate the impurities and fiber. Then the filtrate was allowed to settle for three hours. The supernatant was discarded and the sediment was re-suspended in about five volumes of distilled water for further purification. The starch was allowed to settle for 30 minutes. The procedure of decanting the supernatant and re-suspending the sediment in water was repeated for three to four more times to make sure that the extracted starch was completely free of impurities. Finally the starch was allowed to settle for one hour followed by treating with a solution containing 0.1% NaOH. This step was carried out to reduce the effects of non-starch polysaccharides which may greatly interfere with starch isolation procedure by retaining the smaller granules in the remaining fibrous matrix. A magnetic stirrer was used to stir the viscous solution. The extracted starch was then washed with distilled water to remove the excess NaOH and allowed to dry overnight at room temperature. The resulting starch was stored at -20 °C.

Starch granule observation under optical microscopy (OM)

A few drops of starch granule suspension of each type of tuber were treated with iodine and observed under the optical microscope at ×1000 magnification. As a control, the same observations were made by using starch granule suspensions without treating with iodine. An optical microscope (Carl Zeiss Microscopy GmbH, SN 3150000610) equipped with the camera Zeiss AxioCam ICc 5 was used for the observations, and the captured photos were analyzed using the image analysis software, Zen lite 2.1. The diameter and the cross-sectional area of 20 starch granules were measured for each type of tuber assessed. The data were subjected to ANOVA procedure using the statistical package SAS 9.4 (SAS Institute, NC, Cary, USA).

Starch granule observation under scanning electron microscopy (SEM)

The freshly harvested tubers from each of the six species were used to obtain tissue samples. The tissues were subjected to the vacuum for about 15 min. The vacuum-dried tissue samples were mounted on stubs with carbon tabs. The tissue samples were exposed to gold particles for staining purpose. The observations and the analysis of tissues were done by using the Zeiss scanning electron microscope (SEM) (Jeol SEM 6400, Tokyo, Japan). The images were taken using same magnification, ×1000, as in optical microscope to illustrate the arrangement of starch granules inside the cells. The same procedure was carried out to capture the images of extracted starch granules. Here, instead of tissues, extracted starch granules of all of the tuber types were used to capture the images and, the magnification was adjusted to ×2000 for better resolution.

Assessment of the consumer preference on boiled tubers

The fresh and healthy tubers at the right maturity stage were collected from all six species. The tubers were peeled, washed thoroughly, cut into approximately 3 cm x 3 cm pieces, and boiled in water for 20 mins. The water was filtered out, and the salt was added to ascertain the generally preferred taste. A taste panel of 40 human subjects was employed representing randomly selected equal proportions of males and females in the age range of 18 - 65 years. The required instructions were provided to the panelists before the assessment. Each panelist was given 40 g of boiled tuber pieces of each tuber type for tasting. The panelists were requested to taste and rank the tubers for seven sensory attributes; color, aroma, texture, bitterness, fibrous nature, hardness, and overall taste according to a three-tier scoring system. Score "1" was assigned for the least preferred level. Score "2" was assigned for medium preferred level and Score "3" was assigned for the highest preferred level. The care was taken to refresh the palates of each panelist by providing water in between the tasting each tuber dish (Leighton *et al*., 2010). The ranked data generated by the taste panel were subjected to association analysis using FREQ procedure in SAS.

RESULTS AND DISCUSSION

Morphology of *C. indica*

Canna indica is a perennial herb that grows up to 1-3 m in height at flowering stage (Figure 1). The newly emerged rhizome (Figure 1A) is elliptical in shape and covered with whitish scale leaves. The interior of the newly emerged rhizome is cream-white to ivory in color (Figuers1B and 1C). The fully-grown rhizome is irregular shaped, monopodial or sympodial, stoloniferous or tuberous, branched and also covered with scale leaves where the distal part of the scale leaves are blackish-brown, and proximal parts are whitish-yellow (Figure 1D). However, at maturity, the external color of the rhizome is brown. The root system of *C. indica* consists of an excessively branched network of fibrous roots. The roots do not go deep into the soil; however, forms a mat within 30 cm of soil depth (Figure 1E). The roots are cylindrical and thick with numerous hairs. The color of the roots is creamy white (Figure 1E). As *C. indica* has a rhizome, the roots are adventitious in nature. When rhizomes are separated from the mother plant or in case where mother plant is dead, sprouting of rhizomes happen immediately upon touching with moist soils (Figure 1F). After two weeks,

the shoot gets elongated up to 5 - 15 cm. At this stage, no roots are apparent. In the next three to four weeks, the shoot is extending further, and roots start to grow (Figure 1G). Within another week or so, the first leaf is unfolding and continuing to produce new leaves sequentially (Figure 1H). The plant remains in the vegetative stage for four months and then starts flowering. A potted *C. indica* plant at its fully grown vegetative stage is shown in Figure 1I. The stem is a pseudo stem covered with sheathing leaves. They are erect, herbaceous and cylindrical (Figure 1I). Appearance of the leaves is somewhat similar to the banana leaves; however, the size is less as *C. indica* leaves are 15 – 20 cm wide and 20 – 50 cm long. The leaf shape is more or less ovate. The leaf lamina is dark green with purplish brown margins. Moreover, the leaf lamina is parallel veined and has smooth and wavy margins. The leaf apex is acute (Figures 1J and 1K). Red, solitary flowers are normally 1 cm to 2 cm long and sepals are also having the same length range. In flowers, the reddish-brown coral tube is a prominent character. The flowers are hermaphrodite (Figure 1L). The fruits are spiny, 2 cm to 3 cm long structures consisting of green capsules (Figure 1L). The seeds are 5 – 6 mm long, 2 – 3 mm wide and white color at the initial stage, and black color at the maturity stage (Rao and Donde, 1955). A cultivated *C. indica* which appears as a bush at five months of age is shown in Figure 1M. Figures 1N and 1O show two *C. indica* plants grown in a natural habitat and an infertile land, respectively.

Phylogenetics of *C. indica*

The best substitution model for combined datasets of Prince, (2010) and the *Canna* spp. in Sri Lanka is TPM3uf+I+G (A/C=2.100; A/G=8.260; A/T=1.000; C/G=2.100; C/T=8.260; G/T=1.000). The maxmum convergence for the MCMC tree is at 20 millon chain runs and the ESS value for each prior is above 200. Almost similer branching pattern is obtained for the trees constructed in the baysin and ML frameworks. However, the ML tree resolves the best, thus presented in this study (Figure 2). The *Canna* spp. in Sri Lanka are separately clustered in the Cannaceae clade of the phylogram (Figure 2A). However, the phylogenetic relationships of the Cannaceae clade in this phylogeny are poorly resolved with few polytomic relationships. This has also been described in the Prince, (2010), where they concluded that plastid markers failed to resolve the correct phylogenetic relationships within the Cannaceae family.

The nucleotide variation is recognized in the selected loci of the chloroplast genome of *C. indica* and ornamental *Canna* spp. (Figures 2B, 2C and 2D). Out of the two coding and two non-coding markers employed in the present study, only *rbc*L, *atp*B gene and *trn*H-*psb*A can be effectively employed to identify the nucleotide polymorphism and thereby to designate the species in respective clusters within a UPGMA tree. The marker *trn*L-*trn*F is monomorphic for all the samples sequenced. The informative substitutions in the *atp*B gene are detected; thus an enhanced separation is obtained in the phylogeny (Figure 2B). Accordingly, five edible *Canna* spp. are cladded in the same cluster. Remarkably, the point mutation (A/C) at the 2nd position in *atpB* gene is sufficient to ascertain the variance between the ornamental *Canna* spp. from the edible *Canna* spp. with a genetic divergence of 0.0008. However, the ornamental *Canna* sp. (red flower bearing) is diverged out from the clade which contains the other two ornamental *Canna* spp. (yellow and orange flower bearing). This might be attributed to the synonymous mutations at 1417th and 1418th positions in the *atpB* gene.

Furthermore, five transversions (C/G, C/A, T/G) and one unique transition (A/G) within the *trn*H-*psb*A region are detected. *C. indica* (Kundasale, Matale and Nuwara-Eliya) are grouped into one clade with a unique haplotype, thus clearly differentiating them from ornamental *Canna* sp. (yellow) with a genetic divergence of 0.0014. The clade containing *C. indica* (Nuwara-Eliya) and ornamental *Canna* sp. (red) is distinguishable from *C. indica* (Peradeniya) with a genetic divergence of 0.0014. The ornamental *Canna* sp. (orange) can be considered as the most evolutionary diverged group, by relying predominantly on the SNP variation in *trn*H-*psb*A locus (Figure 2D). Moreover, synonymous mutations are identified in the *rbc*L region. Consequently, *C. indica* (Matale, and Nuwara-Eliya) and ornamental *Canna* sp. (red) are nested in the same clade while *C. indica* (*Nuwara-Eliya*), ornamental *Canna* spp., yellow and orange, are cladded together. Similarly, *C. indica* (Kundasale and Peradeniya) are fallen within the same clade with a 0.0018 genetic divergence (Figure 2C). Based on the results, it can be suggested that the usefulness of the two loci; *trn*H-*psb*A and *rbc*L, in discriminating the ornamental and edible *Canna* species is less, compared with the informativeness of *atp*B gene. The ornamental *Canna* spp. are also identified as *C. iridiflora* and *C. jaegeriana* in the published literature (Prince, 2010; Tanaka *et al*., 2009). However, in the present study for *rbc*L and *trn*H-*psb*A, the ornamental *Canna* spp. do not show any clear separation from *C. indica* (edible Arrow-root) indicating that ornamental *Canna* spp. could also come under *C. indica*. conflicting with the nomenclature provided in (Prince, 2010; Tanaka *et al*., 2009) (Figures 2C and 2D). The ornamental *Canna* spp. and *C. indica* are clearly separated by the polymorphism at the *atp*B gene (Figure 2B) highlighting the possible speciation or sub speciation which requires further studies.

Morphological variability of starch granules

The variability of the size of the starch granules are given in the Table 3. The results revealed that the granule size and the shape are highly variable among the tuber crops studied. *C. indica* starch granules are significantly larger than the granules of the other popular starch crops studied for the comparison purpose. The mean diameter and the mean cross-sectional area of the starch granules are 53.72 µm of 2180.46 µm2 respectively for *C. indica* and they are the largest reported values for the six species studied (P<0.05). The microscopic analysis of the starch granules in the present study further verifies the past observations reporting that *C. indica* has large starch granules (Hermann *et al*., 1997). The smallest size of the starch granules is observed in *I. batatas* (purple) (mean diameter: 10.56 µm, cross sectional area: 91.44 µm2) (P<0.05). The appearance of starch granules under the OM and SEM are presented in

Figure 1: Morphological appearance of the whole plant and the different parts of *C. indica* plant.

A: Newly emerged rhizome; B: Cross section of the newly emerged rhizome; C: Longitudinal section of the newly emerged rhizome; D: Fully grown rhizome at the harvesting stage (economically important part); E: Root system with sprouting rhizomes; F: Close-up view of sprouting rhizome; G: Rhizome sprouted fully and ready to produce leaves; H: Sprouted rhizome with the first leaf; I: *C. india* plant in a pot (a poly bag); J: Adaxial view of a fully grown leaf; K: Abaxial view of the fully grown leaf; L: Flower and fruit; M: A cultivated bush of *C. indica*; N: *C. indica* plant naturally grown; O: *C. indica* plant grown in an infertile land.

Figure 3. A pronounced difference in the shape of the starch granules is observed in *C. indica*. The shape is like a shell of a bivalve with curve-like patterns (scallop-seashell shaped) throughout the surface when viewed under OM (Figure 3A1; 3A2). The granules displayed a disk shape with a smooth surface under SEM (Figure 3A3) and the similar results have previously been reported by Jane *et al*., (1994). However, within the tissue, the cells contain much smaller number of starch granules in *C. indica* in comparison to the other tuber crops studied (Figure 3A4). According to the SEM images and the surface area measurements under OM, the highest surface area is recorded for *C. indica*. Several other studies also have reported the same observation (Wickramasinghe *et al*., 2009, Piyachomkwan *et al*., 2002, Hung and Morita, 2005, Cisneros *et al*., 2009). Moreover, the high viscosity and the ability to make a clear paste of *C. indica* starch make it a good candidate as a thickening agent (Andrade-Mahecha *et al*., 2012).

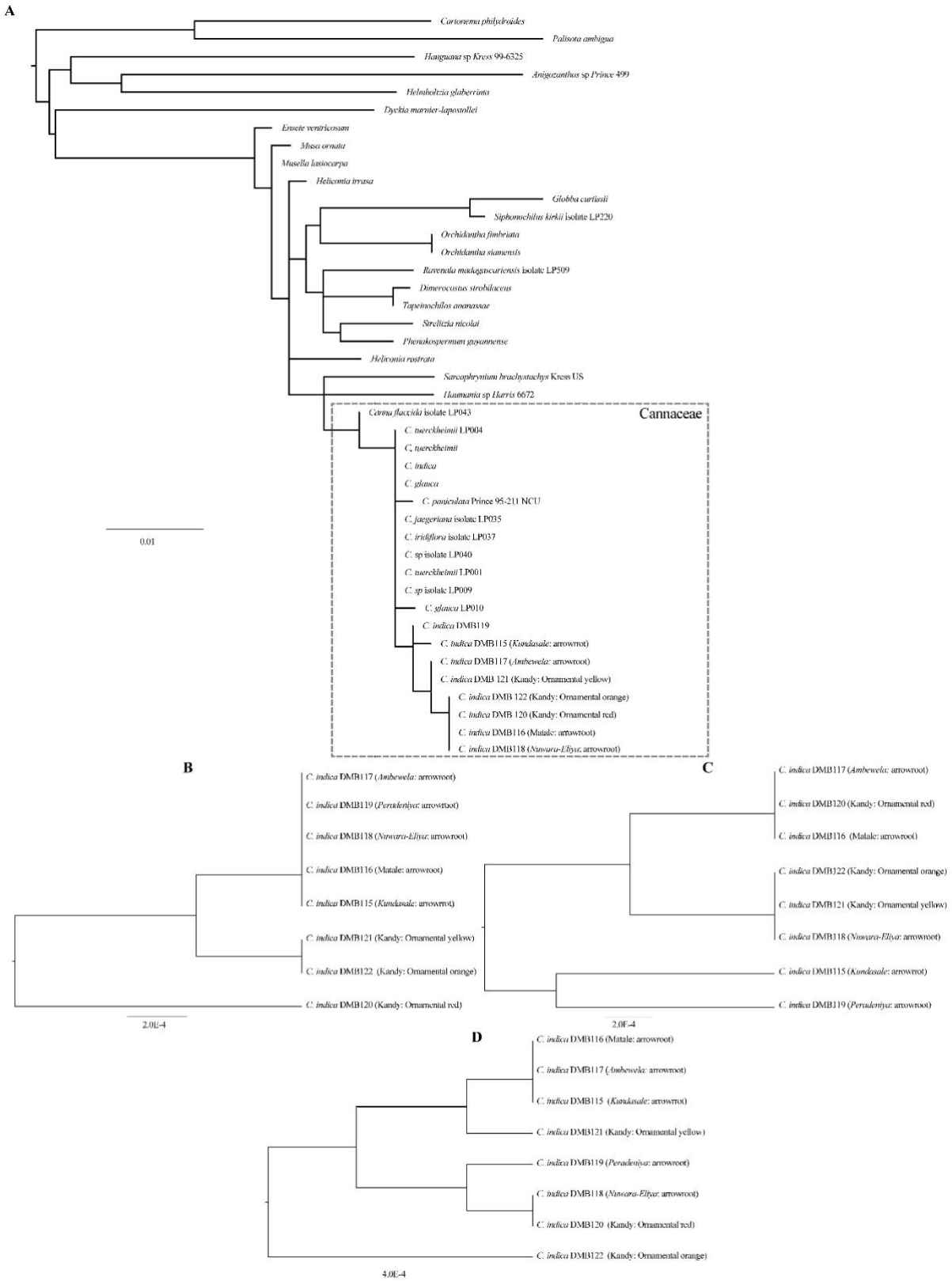

Figure 2: Phylogenetic structure of Canna spp. in Sri Lanka.

A: *rbc*L phylogram showing the phylogenetic position of *Canna* spp. inhabited in Sri Lanka; B: UPGMA tree drawn for *atp*B gene; C: UPGMA tree drawn for *rbc*L; D: UPGMA tree drawn for *trn*H-*psb*A.

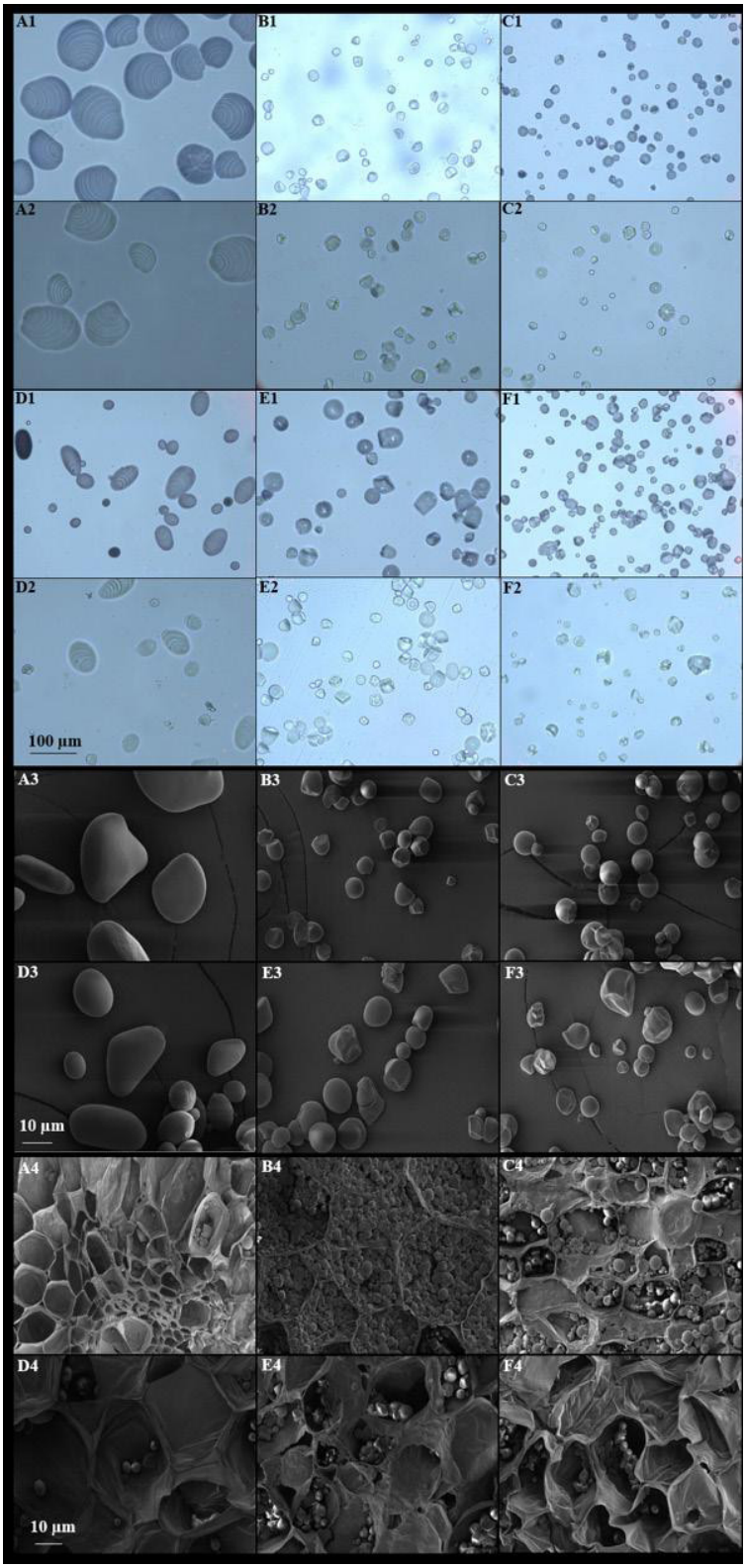

Figure 3: Morphological variability of starch granules in root and tuber crops, observed under the optical microscope and scanning electron microscope.

A: Canna indica; B: *Xanthosoma sagittifolium*; C: *Manihot esculenta*; D; *Solanum tuberosum*; E: *Ipomoea batatas*-purple; F: *Ipomoea batatas*-yellow. 1: Optical microscopic photographs of granules isolated from the tissue and stained with iodine; 2: Optical microscopic photographs of granules isolated from the tissue and not stained; 3: Scanning electron microscopic photographs of granules isolated from the tissue (Magnification: ×2K); 4: Scanning electronic microscopic photographs of granules present within the tissue; (Magnification: ×1K).

Table 3: Variation of the average diameter and the cross sectional area of the starch granules.

Species	Mean diameter (μm)	Mean surface Area (μm2)
Canna indica	53.72a	2180.46a
Solanum tuberosum	14.55c	148.26c
Xanthosoma sagittifolium	15.82c	235.28b
Manihot esculenta	19.86b	322.47b
Ipomoea batatas-yellow	20.01b	313.99b
Ipomoea batatas-purple	10.56d	91.44d

Means denoted by the same letters within the column are not significantly different at P<0.05.

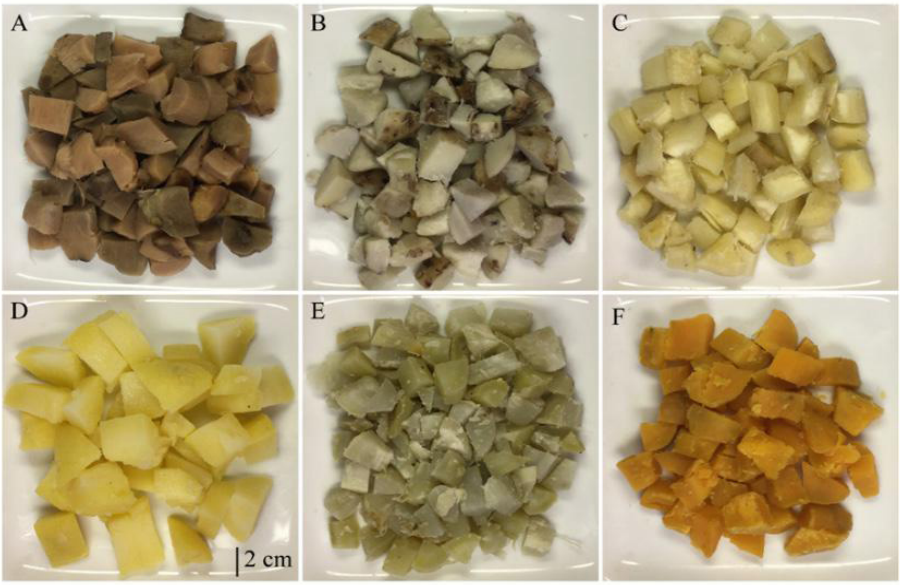

Figure 4: The boiled tuber pieces of the six species used for the taste panel analysis. A: *Canna indica*; B: *Xanthosoma sagittifolium*;C: *Manihot esculenta*; D; *Solanum tuberosum*; E; *Ipomoea batatas* (purple); F: *Ipomoea batatas* (yellow).

In contrast, *X. sagittifolium* is found to possess a significantly high number of starch granules within the tissue with irregular shapes (Figure 3B1; 3B2; 3B3; 3B4). The granules observed under the OM reveals that those of the *I. batatas* (yellow) and *I. batatas* (purple) are similar in morphology with a polygonal shape (Figures 3E1; 3E2; 3F1; 3F2). The exception is being *M. esculenta* and *S. tuberosum* which appears to be having a mixture of shapes (Figures 3C1; 3C2; 3D1; 3D2 respectively). The OM photographs show that some of the *S. tuberosum* granules are oval shaped whereas the others are elliptical (Figures 3D1; 3D2) while SEM displays disk and oval shaped *S. tuberosum* granules (Figure 3D3). The cells of *I. batatas* are observed with spherical and irregular shapes of granules (Figure 3E3; 3F3) and fairly a high number of granules when viewed under the SEM (Figures 3E4; 3F4). However, *M. esculenta* granules possess both spherical and irregular shapes (Figure 3C3). As shown by SEM image, the number of granules within the tissues of *M. esculenta* is higher than that of *C. indica*, however, less than that of *X. sagittifolium* (Figure 3C4). Moreover, *S. tuberosum* displays relatively a smaller number of starch granules within the tissue, nevertheless a high number of granules compared to *C. indica*.

Consumer preference

The representative samples of the boiled tubers prepared for the taste panel are shown in Figure 4. The boiled tubers of *C. indica* are brown in color (Figure 4A) compared to other tubers boiled. All the taste parameters assessed except bitterness are significantly associated with the type of tuber (i.e., tuber species) (Figure 5, $P<0.05$). The strongest association is detected between the type of tuber and the preferred color (Figure 5A). The highest preferred color is recorded for *I. batatas* (yellow) (88%) and *S. tuberosum* (68%). The color of *C. indica*, *M. esculenta* and *I. batatas* (purple) are equally preferred (15-18%). It is interesting to note that the color of *C. indica* is preferred more than that of *X. sagittifolium* which is a more popular tuber crop than *C. indica*. The highest preferred aroma is observed for *I. batatas* (yellow) (28%) followed by *S. tuberosum* (15%). The respondents equally rank *C. indica* and *M. esculenta* for the preferred level of aroma (Figure 5B). The highest preferred texture is reported for the two types of *I. batatas*; however, the texture of *C. indica* is preferred more than *X. sagittifolium* (Figure 5C). The bitterness felt is low for all the tuber types assessed; however, 10 % of the respondents rank the highest felt bitterness for *C. indica* tubers (Figure 5D). The highest felt fibrousness is observed for *C. indica*

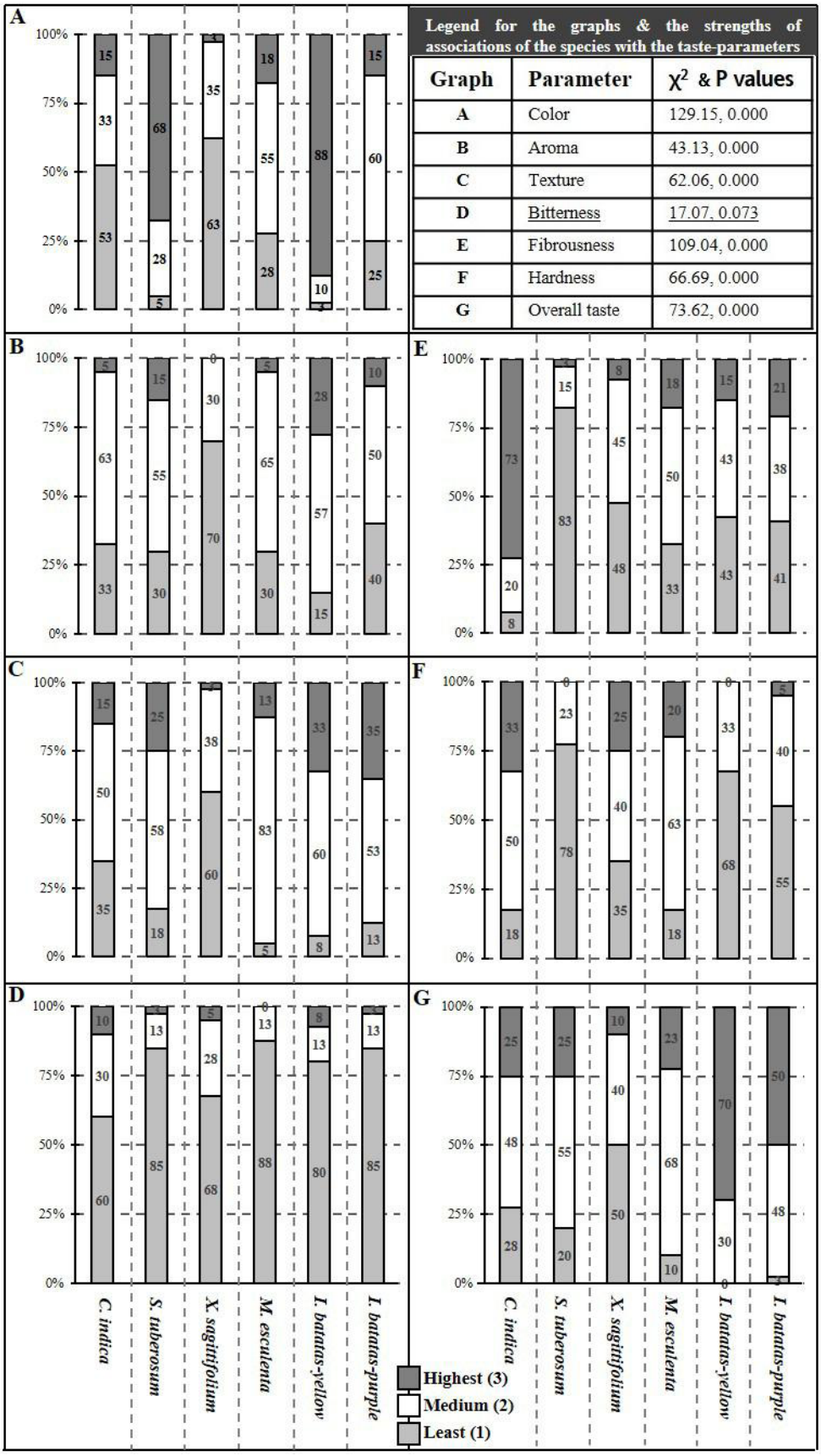

Figure 5: The associations between the taste parameters and six tube crop species studied. Y-axis represent the percentage (%) respondents. The % respondents for each category of association is given within the bars.

(73%) followed by *I. Batatas* (purple) (21%) (Figure 5E). The highest felt hardness is also reported for *C. indica*. The felt hardness is found be the least for *S. tuberosum* and *I. batatas* (yellow). The tubers of *X. sagittifolium* and *M. esculenta* are ranked as the hardest by 25% and 20% respondents respectively (Figure 5F). In the ranking of tubers for overall taste, 70% and 50% of the respondents said they highly prefer the taste of *I. batatas* (yellow) and *I. batatas* (purple) respectively. Interestingly, *C. indica* and *M. esculenta* get equal ranks for the overall taste (Figure 5G).

The association analysis based on the taste panel data provided important insights on the potentials of *C. indica* tubers and the avenues for the crop improvement. It is evident from the analysis that consumers prefer yellow color in boiled tubes but the color of *C. indica* is not entirely rejected. The color of *C. indica* accepted better than that of *X.* sag*ittifolium*. The highest fibrousness and the hardness could be the reasons for the less preference for the tubers of *C. indica*. However, when considering the overall taste, *C. indica* gets equally ranked to the highest levels as the two of the world famous and established tuber crops, *S. tuberosum* and *M. esculenta* (Figure 5G). Thus, improving the tuber properties; softness and less fibrousness, through breeding and selection will undoubtedly uplift *C. indica* from its current underutilized status.

CONCLUSIONS

The phylogenetic tree constructed based on *rbc*L marker revealed that *C. indica* is slightly divergent from the other Canna species in the world. The polymorphism of the *atp*B gene can be used to differentiate *C. indica* from ornamental *Canna* spp. The polymorphism of *rbc*L and *trnH-psbA* cannot be used to differentiate *C. indica* from the rest. The starch granules morphological analysis revealed that *C. indica* has the significantly large (scallop-seashell shaped) starch granules compared to *Xanthosoma sagittifolium*, *Manihot* esculenta, *Solanum tuberosum*, and *Ipomoea batatas*. The consumer preference analysis indicates that boiled *C. indica* tubers are accepted better than that of *X. sagittifolium.* The *C. indica* tubers rate equally to the tubers of *Solanum tuberosum*, and *M. esculenta*. The relatively hardy fibrous nature of the tubers of *C. indica* is the major limiting factor for achieving the highest consumer preference. The breeding programs on *C. indica* must be planned to achieve softer and less fibrous tubers for higher appetite.

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STATEMENT OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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