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Standardization of an efficient DNA isolation protocol in tannia [*Xanthosoma sagittifolium*(L.) Schott]

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Abstract

Genetic variability is very much limited in tannia (*Xanthosoma sagittifolium*(L.) Schott).Morphological variation is limited to colour variation in leaf, petioleand tuber flesh colour in this crop. Tuber shape and size also shows variation. ICAR-CTCRI being the National Repository for tuber crops is maintaining the germplasm collection of edible aroids including tannia. In order to assess the genetic diversity existing in this introduced crop, different DNA isolation protocols were tested in four tannia accessions to identity the best method. CTAB method standardised by Sharma et al., (2008) for taro, DNeasy kit method (Qiagen) and modified Dellaporta method were tried. It was observed that the quality of DNA was good in the modified Dellaporta method with DNA quality ranging from 1.98 (Xa-6) to 2.23 (Xa-67). Good quantity was obtained ranging from 830 ng/μl (ACIX-2) to 1968 ng/μl (Xa-71). The extracted DNA was amenable to ISSR markers. Hence, for all further molecular studies in tannia, the modified Dellaporta method was adopted.

Keywords: Tannia, *Xanthosoma sagittifolium*, DNA isolation, DNA quantity, DNA quality, Nanodrop spectrophotometer

Introduction

Tannia (*Xanthosoma sagittifolium*) is a staple root crop that belongs to the family Araceae. It is a native of tropical, central and South America as well as the Caribbean which is cultivated for its starchy edible tubers. It is a cultivated crop in India, grown for its edible and starchy tubers as well as tender edible leaves. The plant resembles the common taro (*Colocasia esculenta*) in its morphological characters, especially the tubers and the leaves to some extent. In taro, the petiole attachment is in leaf lamina (peltate), whereas in tannia, it is at the base of the lamina (sagittate). Because of its resemblance to taro, which is called cocoyam in some locations, tannia became known as the new cocoyam. (Rubatzky and Yamaguchi, 1997).

The centre of origin for tannia is reported to be tropical America but it is now cultivated throughout Africa, Asia and the Pacific territories. Movement of this crop from its tropical north-eastern south American origin was relatively recent. The crop was taken to Africa during the slave trading era and from there it was rapidly adopted to the other regions and now ranks behind cassava and yams in importance. This crop comes up well under tropical conditions with a mean temperature of above 21°C. In India, tannia is cultivated in the north-eastern states and southern parts of the country (Das et al., 2016). The crop is also amenable to organic manures (Suja et al., 2009).

A herbaceous perennial, *X. sagittifolium* has a corm or main underground stem in the form of a rhizome from

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which swollen secondary shoots, or cormels sprout. Several large leaves also sprout from the main stem, which are sagittate and erect with long, ribbed petioles; inflorescences sprout between the leaves in a spadix, with a white 12 to 15 cm spathe which closes at its base in the form of a spherical chamber and opens at the top into a concave lamina; the spadix is cylindrical, slightly longer than the spathe, with female flowers on the lower portion, male flowers on the upper portion and sterile flowers in the middle portion. The spadices are rarely fertile and produce few viable seeds.

The corm, cormels and leaves are the main economically important parts of the plant. The main corm (mother corm) is acrid and hence only the daughter cormels (side corms) are consumed. The cormels which are consumed as food contains 17-26% carbohydrate, 1.3-3.7% protein and 65-77% water and have nutritional value comparable to potato (Onwueme and Charles, 1994; Agueguia, 2000). The carbohydrate part is mostly composed of starch which is relatively large grains with an average diameter of 17-20 μm (Onwueme, 1978). This makes tannia starch, less readily digested than that of taro. Like taro, the corms of tannia contains raphides and should not be fed to human or livestock without cooking. *X. sagittifolium*is used as a medicinal species both as food and medicine to prevent and treat bone diseases, such as osteoporosis, in traditional Brazilian medicine (de Oliveiraet al., 2012).

Mostly owing to its introduced nature, genetic variation is very limited in this crop cultivated in India. Morphological variation is seen in the colour of leaf, petiole and tuber flesh. Tuber shape and size also shows variation. Majority of the collections present in the National Repository maintained at ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI) have green colour petiole whereas, a few have purple colour too. According to Sepúlveda-Nieto et al. (2017), there are some distinctive morphological markers for identification of tannia such as: leaves with subcoriaceous textures, basal insertion of the petiole, green pseudo-stem in the basal portion with exudate being white and the presence of two collector veins. However, due to their similar phenotypic characteristics, especially in the form and colour of the leaves and petioles, *X. sagittifolium* (tannia) and *C. esculenta* (taro) are popularly mistaken for one another (Onwueme, 1999; Onyeka, 2014). In such cases, molecular studies have been useful in identifying the genetic differences between species which closely resemble one another. Within the realm of molecular markers, Inter-Simple Sequence Repeat (ISSR) markers have proven to be highly effective in the discrimination of diverse taxa. To initiate molecular studies in tannia, different DNA isolation protocols were tested using four tannia accessions to assess the suitability of the method in obtaining good quality DNA for studying the genetic diversity existing in this introduced crop.

Materials and Methods

Source of sample

The study was carried out at the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram. Four accessions of *Xanthosoma sagittifolium*(tannia) conserved and maintained in the ICAR-CTCRI National repository were selected for the present study (Table 1). The plants were raised in the field and young leaves from 5–6 month-old plants were used for DNA isolation.

Sl. No.	Accession name	Species name	Place of collection
1	Xa 6	*X. sagittifolium*	Unknown
2	Xa 71	*X. sagittifolium*	Unknown
3	ACIX 2	*X. sagittifolium*	Kerala
4	Xa 67	*X. sagittifolium*	Kerala

DNA extraction methods

Three DNA isolation methods viz., CTAB method standardised by Sharma et al., (2008) for taro, DNeasy kit method (Qiagen) and modified Dellaporta method were tried. Modified CTAB method standardised for taro (Sharma et al., 2008). The young leaves from 5-6 months old tannia plants were collected and weighed 1.5 g avoiding the mid rib regions and veins and crushed in liquid nitrogen. The extraction buffer consisting of 100 mM tris-HCl (pH 8), 20 mM; EDTA (pH 8), 2 M NaCl, 2% CTAB (w/v), 2% PVP (Mw 40,000), 2%b-mercaptoethanol (v/v) was prepared. Freshly prepared buffer was preheated at 65°C, added to the above powder and gently homogenized. The homogenate was transferred to 2 ml microfuge tubes, labelled and kept in a water bath at 65°C for 45 min with intermittent shaking. The samples were then centrifuged at 12,000 rpm for 10 min.The supernatant was added with equal volume of 24:1 (v/v) chloroform/isoamyl alcohol andagain centrifuged at 12,000 rpm for 10 min. The supernatant was taken and 5 μl of RNase A (10 mg ml^{-1}) was added and mixed gently by inversion. The samples were then incubated in a dry bath at 37°C for 45 min and added with equal volume of chloroform: isoamyl alcohol (24:1) and centrifuged at 12,000 rpm for 10 min. The upper aqueous layer was taken and treated with 2/3 volume of iso-propanol. The precipitated DNA was pelletized by centrifuging at 10,000 rpm for 15 min. and then washed with 70% ethanol by spinning at 10,000 rpm for 5 min. The DNA pellets were air dried to remove any traces of ethanol followed by suspension in 50-100μl of 1X TE buffer. The DNA suspension was stored at -20°C for further use.

DNeasy® plant mini kit (Qiagen) method

The leaf samples were disrupted using a mortar and pestle. Then the protocol given in the kit was followed.

Added 400 µl buffer AP 1 and 4 µ RNase A, vortexed and incubated for 10 min. at 65^{0}C. The tubes were inverted 2-3 times during incubation and 130 µl buffer P 3 was added, mixed and incubated for 5 min. on ice. The lysate was centrifuged for 5 min. at 14,000 rpm and pipetted into a Q I A shredder spin column placed in a 2 ml collection tube and centrifuged for 2 min. at 14,000 rpm. The flow-through was transferred into a new tube without disturbing the pellet, if present. Then 1.5 volumes of buffer AW 1was added, and mixed by pipetting. The mixture (650 µl)was transferred into a DNeasy mini spin column placed in a 2ml collection tube and centrifuged for 1min. at 8000 rpm., the flow-through was discarded. This step was repeated,and the spin column was placed into a new 2 ml collection tube. Then, 500 µl buffer AW 2 was added and centrifuged for 1min. at 8000 rpm. The flow-through was discarded. Then added another 500 µl of buffer AW 2, centrifuged for 2 min at 14,000 rpm. and transferred the spin column to a 1.5 ml or 2 ml micro centrifuge tube. Added another 100 µl buffer AE for elution. The mixture was incubated for 15 minutes at room temperature. Then centrifuged for 1 min at >600 × g. This step was repeated, and the eluted DNA was stored at -20°C.

Modified Dellaporta method (Dellaporta et al., 1983)

One g of leaf bits were taken from the young tender leaves and were transferred into a pre-chilled mortar and pestle, frozen already using liquid nitrogen and ground to a fine powder. The powdered samples were mixed with 15 ml of extraction buffer containing 100 mM tris-HCl (pH 8), 20 mM; EDTA (pH 8), 2 M NaCl, 2% PVP (Mw 40,000), 0.2% ß-mercaptoethanol (v/v) was added and kept at 4°C. To this mixture, 1ml of 20% SDS was added, thoroughly mixed, and incubated at 65°C for 1 h in a water bath. Five ml of 5M potassium acetate was then added and kept on ice for 20 min. and centrifuged at 12,000 rpm for 20 min. and the clear aqueous phase was transferred to a new sterile tube. Equal volume of ice-cold isopropanol was added and mixed gently by inversion and then kept in the freezer until DNA was precipitated out. Centrifugation was performed at 12,000 rpm for 10 min., the pellet obtained was dissolved in 500 µl sterile double distilled water and transferred to a microfuge tube. Five µl of RNase A (10 mg ml^{-1}) was added and incubated at 37°C for one h. Five hundred µl of 24:1 (v/v) chloroform:isoamylalcoholwas then added, mixed well and centrifuged at 12,000 rpm for 15 min. The supernatant was collected, added with two volumes ice cold absolute ethanol and 1/10volume 3M sodium acetate and kept overnight incubation or one h in -20°C. It was centrifuged at 12,000 rpm for 10 min. and the supernatant was discarded. To the pellet, 500 µl of 70% ethanol was added to wash the DNA. Then the alcohol was discarded, and the DNA was air dried fully. The DNA was then dissolved in 500 µl of TE buffer and stored the sample at -20°C.

Qualitative and quantitative analysis of extracted DNA

The DNA yield was measured by using a nanodrop spectrophotometer (Denovix DS-11+) at 260 nm. The DNA purity was determined by calculating the absorbance ratio A260/280. For quality and yield assessments, electrophoresis was done of all DNA samples in 1% agarose gel, stained with ethidium bromide and bands were observed in gel documentation system (Alpha Innotech).

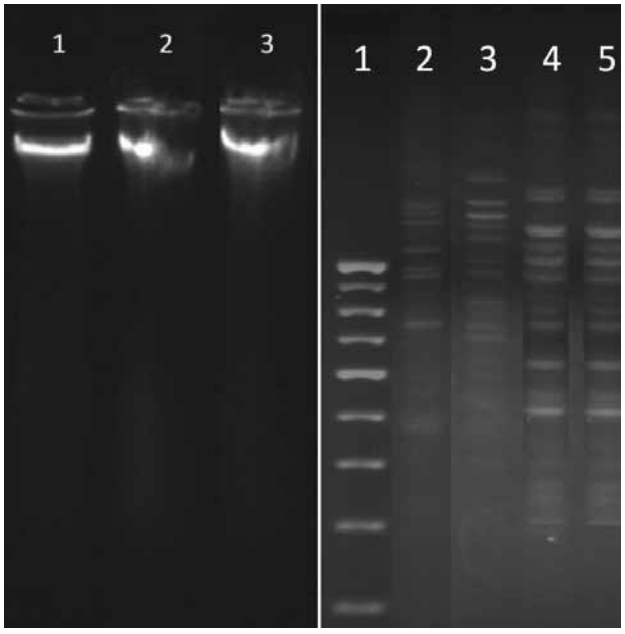
Inter Simple Sequence Repeat (ISSR) study

The PCR amplification reaction was carried out with nine ISSR markers in a 20 µl reaction volume containing 10 mM Tris-HCl, pH 8.3, 20 mM $MgCl_2$, 1 mM dNTP mix, 0.2 µM of each primer, 1 U of Taq DNA polymerase, and 10 ng of template DNA. ISSR-PCR was performed in a thermal cycler (PTC-100tm MJ Research Inc., USA) for 35 cycles consisting of denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec, and extension at 72°C for 2 min. The final extension was carried out at the same temperature for 5 min. The amplified product was checked in 2% agarose gel electrophoresis and bands were observed in gel documentation system (Alpha Innotech).

Results and Discussion

Fresh young leaves were used for the isolation of goodquality DNA as mature leaves contain higher quantities of polyphenols and polysaccharides (Porebski et al., 1997), making it difficult to isolate DNA of good quality. The isolation of pure, intact, and high-quality DNA is a very important step for any molecular studies. DNA isolation methods need to be adjusted to each plant species and even to each plant tissue because of the presence of metabolites in them, unlike animals and microbes (Sangwan etal., 1998). In the present study, three different DNA isolation protocols were tested. The quality and quantity of DNA was tested using a Nanodrop spectrophotometer and agarose gel electrophoresis (1%), respectively to ensure the use of good quality of DNA for molecular marker studies. Among all the tested protocols, the modified Dellaporta method yielded good results. The modified CTAB extraction and Qiagenkit method did not show promising results for the four tannia accessions as evidenced by the sheared band in the agarose gel (Fig. 1a).

The quantity of DNA present in each sample as determined by the 260/280 value using a nanodrop spectrophotometer are shown in Table 2. Good quality DNA was obtained from the four accessions studied and it ranged between 1.98 (Xa-6) to 2.23 (Xa-67). DNA readings of 1.8 - 2.0 are considered very good



(a) (b)

Fig. 1. (a) DNA isolated using the different protocols in tannia accession Xa-67 (Lane1: modified Dellaporta method; Lane2: Sharma et al., 2008; Lane3: DNeasy Qiagen kit method), (b) ISSR pattern of tannia accessions (Lane1: 100 bp ladder; Lane2 to Lane5: tannia accessions)

for molecular studies. Washing with Chloroform: isoamylalcohol treatment ensures removal of chlorophyll, pigments, and dyes (Sahu et al., 2012) thus improving the quality of DNA. The above method yielded good quantity ranging from 830 ng/μl (ACIX-2) to 1968 ng/μl (Xa-71). In the modified Dellaporta method, the pre-cooling of the mortar and pestle, use of ice cold isopropanol and refrigeration steps had a positive effect on the DNA extracted. Here, SDS was used instead of CTAB. Addition of potassium acetate served the purpose of facilitating the removal of a significant portion of the proteins and polysaccharides within the sample, forming a complex with the insoluble potassium dodecyl sulphate precipitate (Dellaporta et al., 1983).

Table 2. Quantity and quality of DNA isolated using the modified Dellaporta method as assessed using nanodrop spectrophotometer

Sl. No.	Accession name	OD 260/280	Concentration (ng/μl)
1	Xa 6	1.98	1219
2	Xa 71	2.06	1968
3	ACIX 2	2.21	830
4	Xa 67	2.23	976

It was noted that the extracted DNA was amenable to the ISSR markers tested. Clear banding patterns were observed in the ISSR study (Fig.1b) and hence, this modified Dellaporta method was employed for tannia.

Conclusion

The study's findings led to the conclusion that for obtaining high-quality DNA, the modified Dellaporta method proved effective and was also suitable for ISSR marker analysis, resulting in distinct and consistent bands. Therefore, this method is recommended for the isolation of genomic DNA from tannia.

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