



Evaluation and Characterization of High Yielding Cassava Mosaic Resistant Variety YTP2

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The cassava variety YTP2 (Me 681) has been developed through selection from Thondamuthur type at Tapioca and Castor Research Station, TNAU, Yethapur. The performance of YTP2 in the Adaptive Research Trial (ART) and On Farm Trial (OFT) in the farmer's field inferred that this new variety is well adapted to cassava growing districts of Tamil Nadu. In addition to the above, YTP2 was found to be resistant to cassava mosaic disease incidence (CMD). Plants are erect, medium growing and non-branching type and suitable for growing under irrigated and rainfed conditions. The internodal length is shorter and the leaf size is medium with sufficient canopy. The leaves of the plants droop down to reduce the transpiration loss which is more advantageous to overcome or escape from drought and heat stress during summer season. It is a dual purpose variety wherein the tubers contain high starch content which is much favourable for the manufacture of starch, sago and also suited for table purpose. The overall performance of this variety showed higher tuber yield (42.20 t ha⁻¹) and starch content (28.40%) which is 15.94% and 18.20% increase over the check varieties YTP1 and H226 respectively. The results of DNA fingerprint data involving SSR markers (SSRY235, NS169 and NS928) showed that it is genetically distinct from the existing commercial varieties viz., YTP1, H226 and Sree Athulya.

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1. INTRODUCTION

Cassava is a potential starch yielding crop belonging to the family *Euphorbiaceae* and native to South America. The leading cassava production countries are Brazil, Indonesia, Nigeria, Thailand, India and the Democratic republic of Congo. In India, cassava was introduced by the Portuguese during the 17th century. The edible tuberous root of cassava serves as a staple food in the tropics. Cassava is mostly grown in resource constraints with limited irrigation, making it an important food security crop [1]. Cassava flour is gluten-free and hence it attracts consumers for practising gluten-free diet. In India, Cassava is cultivated in an area of 1.6 lakh ha with the production of 4.9 million tonnes [2]. This crop is cultivated mainly in Southern Peninsular region, particularly Kerala, Tamil Nadu and Andhra Pradesh accounting for 93% of area and 98% of production in the country. The varieties with high tuber yield and starch content are very much preferred by the sago and starch manufacturing industries [3]. In Tamil Nadu, Cassava is cultivated both under irrigated and rainfed conditions involving the popular varieties viz., Mulluvadi1, H226, H165, CO2, CO3, CO(TP)4, Sree Jaya and Sree Harsha etc., which are poor tuber yielding with less starch content and highly susceptible to the incidence of cassava mosaic disease (CMD) [4,5].

Under these circumstances, there will be shortage in supply of cassava in the ensuing years, since the potential demand will overcome the potential supply by 3.047 and 2.758 million tonnes [6]. With increase in demand of cassava both for industrial and table purposes, it is imperative to evolve new varieties with high tuber yield, high starch content and resistance to cassava mosaic disease. Hence, in the present study an attempt was made to evolve suitable cassava variety with higher tuber yield, starch content and resistance to CMD incidence.

Further the comparative evaluation of the genetic profile of the already released varieties along with the newly developed variety is necessary to assess the genetic diversity and dissimilarity among the genotypes. Conventionally, breeders employ phenotypic characteristics for varietal identification and varietal grouping [7]. DNA based markers like SSR and SNPs are gaining importance as an alternative to phenotype based varietal identification [8]. Molecular markers are

using the advantage of allelic diversity among varieties for differentiation and SSR proves to be a quick alternative for the GOT done to test genetic purity [9]. In the present study, SSR allele diversity between cassava varieties developed at TNAU were analysed to develop variety specific fingerprint for varietal identification.

2. MATERIALS AND METHODS

The YTP2 (Me681) is a promising culture selected from the genetic resource available at Tapioca and Castor Research Station, Yethapur based on the continued yield performance over the years. The station trials were conducted for the culture (Me681) along with the commercially available check varieties at TCRS, Yethapur for the performance of yield, starch content, dry starch yield and CMD incidence at harvest from 2014 to 2016. The Multi Location trials were conducted for a period of two years from 2017 to 2019 in different regions of Research Stations of TNAU.

The first year of MLT was conducted between 2017 and 2018 at Regional Research Station, Paiyur, Centre of Excellence for Millets, Athiyanthal, Pattukottai, Regional Research Station, Vridhachalam, Vegetable Research Station Palur, Horticultural College and Research Institute, Coimbatore, Tapioca and Castor Research Station, Yethapur and Agricultural Research Station, Bhavanisagar for yield and starch content. The second year MLT trial was conducted during 2018 - 2019 at all the centres as in first year. The Adoptive Research Trials were carried out between 2019 and 2020 for yield, starch content and CMD incidence at harvest in the farmer's holding in the districts of Salem and Namakkal. Apart from the above, the large scale On Farm Trials were conducted for one year (2019-20) in progressive farmer's fields of Salem and Dharmapuri districts. For assessing its performance the key parameters viz., plant height, stem girth, number of tubers, yield per plant, starch content and CMD incidence were recorded. The statistical analysis of the yield and quality parameters was done by adopting the statistical procedures suggested by [10] and the critical difference was worked out at five per cent (0.05) probability. The pooled mean analysis was carried out with AGRES software package and MS Excel spreadsheet.

The mean performance of YTP2 was compared with the check varieties for the yield, starch content and CMD incidence at the time of harvest. The standard package of practices recommended by [11] was followed during the phase of evaluation.

2.1 Morphological Traits

The phenotypic performance of the varieties was evaluated by morphological characterization. The DUS characters for the qualitative traits namely petiole colour, orientation of the petiole, predominant shape of the central leaf lobe, leaf colour, leaf vein colour, young stem colour, plant type, plant branching habit, tuber shape, tuber skin colour, tuber rind colour, tuber flesh colour and the quantitative trait namely predominant number of leaf lobes were recorded.

2.2 Estimation of Starch Content

A rapid titrimetric method standardized at Central Tuber Crops Research Institute was followed for the quantification of starch in fresh tubers [12]. The starch content of the sample was calculated by using the formula,

$$\text{Starch (g/100g fresh weight)} = \frac{10^a \times 100^b \times 0.9^c \times 100}{t \times 2^d \times 1000}$$

Where, 'a' denotes the titre obtained for ferricyanide reagent, while calibrating against standard glucose solution, 'b' is the total volume of starch hydrolysate, 'c' is the Morris factor for

converting sugar to starch, 'd' is the weight of tuber samples in gram used for analysis and 't' is the titre value for starch hydrolysate.

2.3 Scoring of CMD Incidence

Cassava Mosaic Disease (CMD) incidence was calculated by using the formula,

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

The scoring of cassava mosaic disease incidence was done based on the different grades of infestation suggested by International Institute of Tropical of Agriculture [13]. There are five grades of CMD incidence viz., grade 1 indicates no symptom, grade 2 indicates mild symptoms with minimal distortion of leaves and grade 3 shows pronounced mosaic pattern with narrowing and distortion of leaves and grade 4 reveals severe mosaic distortion and reduction in leaf size and grade 5 indicating very severe mosaic symptoms (Fig.1).

2.4 DNA Extraction

The DNA extraction of the samples was carried out by following the procedure designed by [14]. The DNA samples were quantified by using a Fluorometer (DyNA Quant TM200, M/s Hoefer Pharmacia, Biotech Inc., USA) and its quality was checked using 0.8% agarose gel and the final DNA concentration of individual samples was adjusted to 25ng/μl.



Fig. 1. Different grades of cassava mosaic disease infestation

2.5 Polymerase Chain Reaction

The SSR markers viz., SSRY50, SSRY235, SSRY332, SSRY303, NS169 and NS928 were used to analyse the allele differentiation among the varieties. The PCR reaction was performed in volume of 20µl containing 5ng genomic DNA, 0.2µM of each forward and reverse primer, 10mM Tris-HCL (pH 7.2), 50mM KCL, 1.5mM MgCl, 20µM of each dNTPs, 0.3U *Taq* DNA polymerase and autoclaved nano pure water. The DNA amplification of all the samples were carried out on a PTC100 (M/s MJ Research Inc.) Thermo Cycler was programmed for initial denaturation of 5 min. at 94°C then 35 cycles of 1min. at 94°C, 2 min. at 56°C, 2 min. at 72°C and a final extension of 5 min. at 72°C. Denatured PCR product of volume 3 µl was subjected to 6% PAGE (Polyacrylamide Gel Electrophoresis) for 2 hours at 100 W and DNA was visualized by silver staining in line with Promega's silver staining protocol.

3. RESULTS AND DISCUSSION

Successful breeding programme starts with the collection, characterization and evaluation of germplasm. Selection is an important breeding tool for the vegetatively propagated crops like cassava and more useful when the phenotypic characters are having low heritability. Cassava is monoecious and outcrossing by protogyny mechanism leads to high degree of

heterozygosity. Subsequently, these heterozygous individuals are propagated vegetatively to maintain the genetic characters. Selection process is based on the acquiring of additive effects especially efficient for traits with high heritability when there is a broad genetic base. The selected cultures/accessions have to be rigorously tested in the local environmental conditions along with the check varieties. The outperformed cultures will be notified and released as variety. In recent days, many of the new varieties of cassava is being developed and evaluated with improved qualities like tuber yield and starch content in many parts of world. Clonal evaluation based on the use of selection index is an accurate measure for the development of commercial varieties [15]. The variety YTP 2 is identified from the cassava genetic stock maintained at Tapioca and Castor Research Station, Yethapur based on the desirable phenotypic characters of the plants. The setts (stem cuttings) of the chosen plants were propagated and multiplied to the next generation (Fig. 2).

During the process of varietal development and evaluation, a total of 105 trials were conducted for evaluation of performance of YTP2 (Me 681) with the check varieties. The mean data under MLT, ART and OFT are presented in Figs.3, 5 and 6. Significant difference in the performance of YTP 2 in terms of tuber yield, starch content, starch yield and CMD incidence was observed.

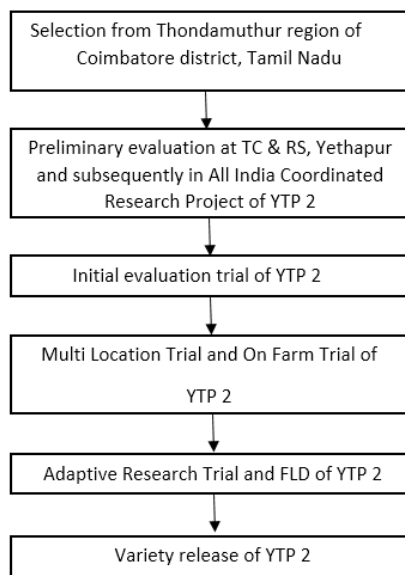


Fig. 2. Schematic diagram showing the development and release of cassava variety YTP 2

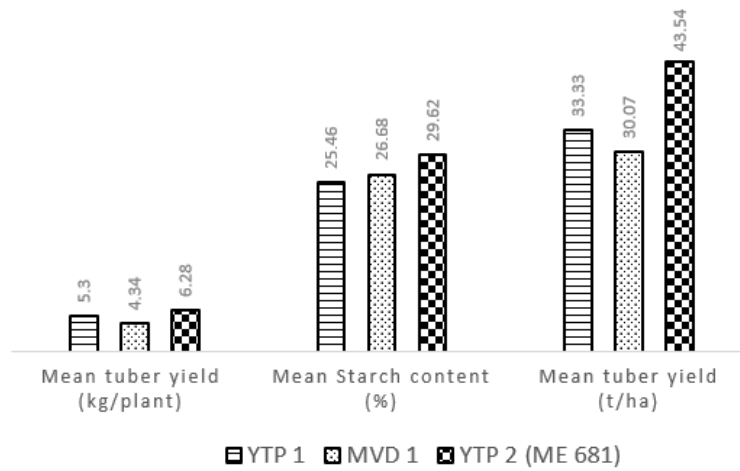


Fig. 3. Mean tuber yield (kg/plant), starch content (%) and tuber yield (t/ha) of the cassava varieties



Fig. 4. Tubers of Cassava variety YTP-2

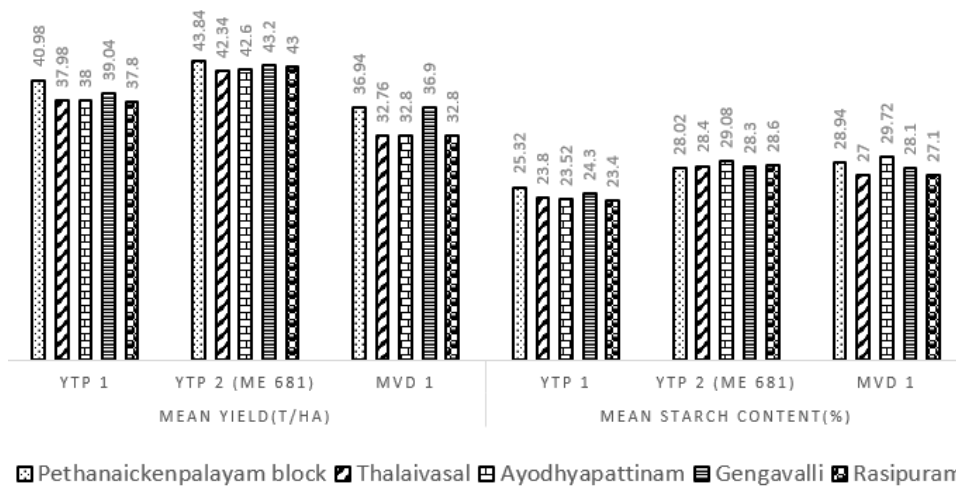


Fig. 5. Tuber yield (t/ha) and Starch content (%) of the cassava varieties in ART trials

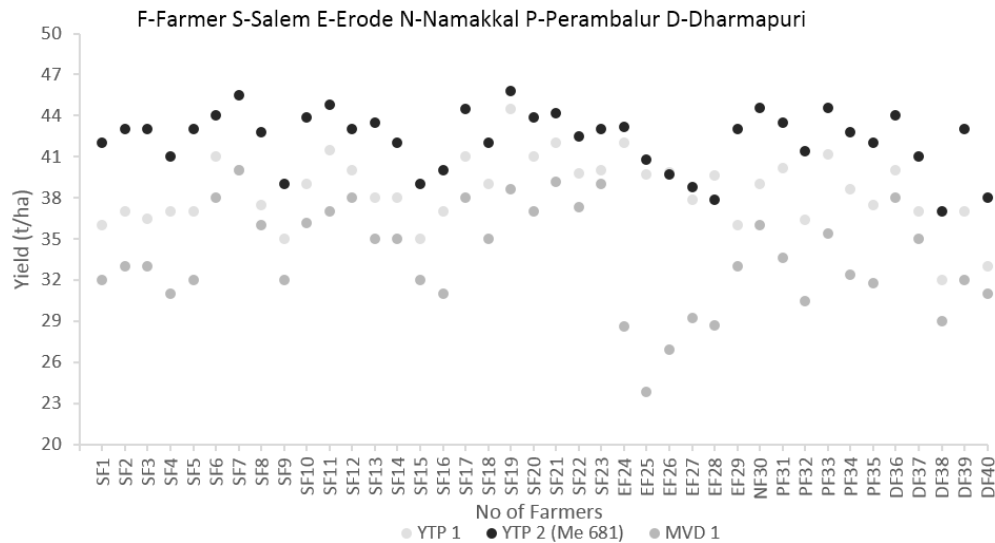


Fig. 6. Tuber yield (t/ha) in farmer's field in OFT

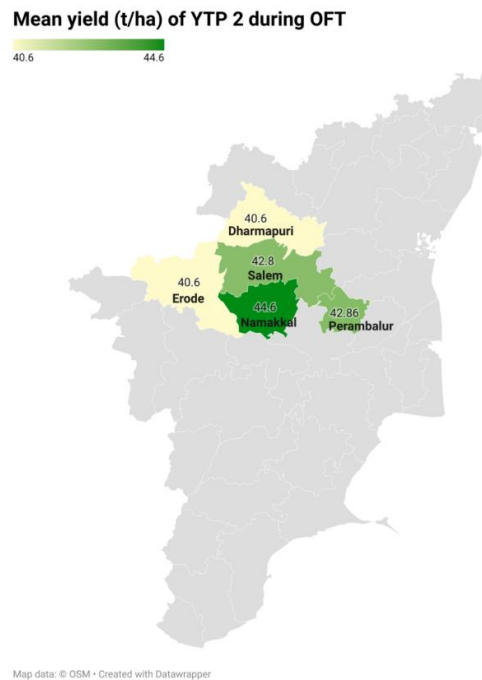


Fig. 7. Yield (t/ha) of YTP 2 during OFT

The overall performance of YTP2 in different trials in terms of tuber yield and starch content was compared and presented in Table 1. The maximum mean tuber yield (42.2 t/ha) and starch content (28.4%) was observed in YTP2 and in the check variety, YTP1 minimum tuber yield (36.6 t/ha) and starch content (24.0%) was recorded. Consequently, based on the

outstanding performance in all the trials, YTP2 exhibited 15.94% increase in tuber yield and 18.20 % increase in starch content. The heterozygous nature of the cassava in breeding makes it difficult to identify the plant with good agronomic traits. The results of the present investigation are in line with the findings of [15,16,4].

Table 1. Overall performance of the cassava YTP2 (Me 681) for tuber yield (t/ha) and for starch content (%)

Particulars	No of trials	Tuber yield (t/ha)			Starch content (%)		
		YTP 1	YTP 2	H 226	YTP 1	YTP 2	H 226
TCRS, Yethapur	8	42.8	45.2	37.4	23.5	29.8	28.0
MLT	32	33.3	43.5	30.9	25.4	29.6	27.7
ART	25	38.4	43.0	33.8	23.8	28.6	27.8
OFT	15	38.4	41.8	30.8	23.9	28.6	26.9
Farmer's field	25	38.5	42.6	35.5	24.5	28.4	27.3
Weighted Mean	-	36.4	42.2	32.5	24.0	28.4	27.0
Per cent increase over check variety YTP 1	15.94				18.20		

Table 2. The morphological features of YTP1 Vs YTP2

SI.No	Character	YTP1	YTP2
1	Petiole colour	Greenish purple	Reddish cream
2	Orientation of petiole	Inclined upward	Inclined upward
3	Predominant shape of central leaf lobe	Pandurate	Pandurate
4	Predominant number of leaf lobes	Nine lobed	Seven lobed
5	Leaf colour	Dark green	Light green
6	Leaf vein colour	Green	Green
7	Young stem colour	Dark green	Whitish green
8	Plant type	Erect	Erect
9	Plant branching habit	Non branching	Non branching but branches at top
10	Tuber shape	Medium, cylindrical, Tuber neck absent	Long, cylindrical
11	Tuber skin colour	Brown	Pinkish white
12	Tuber rind colour	Light cream	Pink
13	Tuber flesh colour	White	White



Fig. 8. Comparison of YTP1 and YTP2 (Me 681)

3.1 Special Features of YTP2

The YTP2 has been assigned with the national identity IC634991 by registering in National Bureau of Plant Genetic Resources and it was notified for commercial cultivation by the Ministry

of Agriculture and Farmers Welfare, New Delhi, India. Comprehensive characterization of the varieties based on the agronomic traits is a necessary prerequisite for the commercial cultivation. The variation in morphological traits of YTP1 and YTP2 are presented in Table 2 and

Fig. 8. Plants are erect, medium growing and non-branching type and suitable for growing under irrigated and rainfed conditions. Stem is greenish white with short internodal length and more number of leaves with sufficient canopy. It has been observed that thin lobes of the leaves are considered as a dominant trait when compared to wider lobes of the leaves. Similarly, brown colour of the root skin is dominant over white skin [17]. Vegetative stage of the crop coincides with early summer and during summer, the leaves droops down and reduces the transpiration loss which makes the plant to overcome the drought and heat. The tubers are long, cylindrical with pinkish white skin. The tuber flesh is white in colour which is highly suited for the preparation of starch and sago. The leaves are completely free from CMD incidence.

3.2 CMD Incidence

Cassava Mosaic Disease (CMD) is one of the important production constraints in the commercial cultivation of cassava [18]. This the most devastating diseases of cassava leading to the distortion of leaves resulting in poor photosynthetic activity which leads to the greater loss in tuber yield and reduction in starch content. Throughout the phase of varietal evaluation, YPT2 was completely free from CMD incidence until the time of harvest, whereas the YTP1, MVD1 and H226 had severe CMD incidence which reflected on the lesser tuber yield and starch content. Roguing of CMD infected plants and selection of disease free planting materials for the commercial cultivation is considered to be more tedious and [19]. The results of the present findings are in

corroboration with the findings of [20,21,22] wherein the drastic selection procedure is necessary to eliminate genotypes susceptible to CMD. Hence, there is urgent need for building resistance to invasive pests and diseases more specifically for cassava mosaic disease (CMD).

3.3 SSR Analysis

Isozyme analysis has been successfully exploited for the study of genetic variation in cassava [23,24,5]. Six SSR primers were used to study the allele differentiation in YTP2, Sree Athulya and YTP1. All the primers were amplified in the three samples under study.

SSRY50, SSRY235 and SSRY332 amplified as single band in YTP2, whereas SSRY303, NS 169 and NS 928 amplifications of YTP 2 produced double bands. Out of the six primers, SSRY332 and SSRY303 showed identical allelic pattern in both the control samples. Similarly, NS 169 and NS 928 showed similar allelic pattern in YTP2 and YTP1 with varying allele size.

The primers produced two banding patterns namely single band and double bands. Primer amplification in YTP2 exhibited unique allele size in comparison to Sree Athulya and YTP1 for all the SSR primers used. SSRY235, NS 169 and NS 928 amplification produced unique allele size in all the three samples (Fig. 8). Above findings are in line with the findings of [25]. Similarly tagging of genes with agronomic trait of interest is more efficient for selection programmes and the dissection of quantitative variations [26] and [27].

Table 3. List of SSR primers employed to study allele differentiation among YTP2, Sree Athulya and YTP1

Locus	Forward Primer (5'-3')	Reverse Primer (5'-3')	Product size (bp)		
			YTP 2	Sree Athulya	YTP1
SSRY50	CCGCTTAACTCCTT GCTGTC	CAAGTGGATGAGCTAC GCAA	260	258+294	294
SSRY235	CAGCTTTGCCATCC AATTTT	CAGCAAATGACATGA GTGTATCTC	205	230	245+256
SSRY332	CAGGCTCGAGGTC TTCTTTG	CCACCCCATCTTCAACA TTT	232	245+258	245+258
SSRY303	GCATCATCACCATT TTTCTTTG	TGGAAGGTGTTAGCAA CTGTG	198+210	189	189
NS 169	GTGCGAAATGGAAA TCAATG	GCCTTCTCAGCATATG GAGC	230+240	218	235+246
NS 928	GATACCCACAAGCC CAAAG	GACCCACCCATCCACT AGAA	202+212	196	210+222

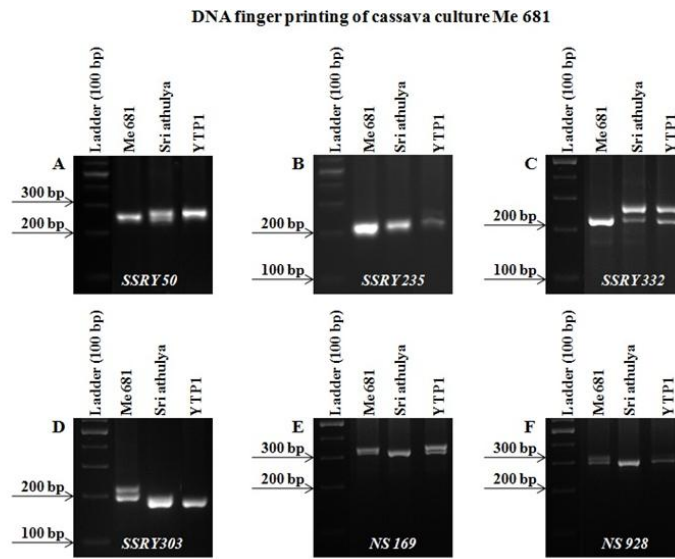


Fig.9. SSR marker segregation profile of YTP2 (Me 681), Sree Athulya and YTP1 with arrow marks indicating the allele size in base pairs

4. CONCLUSION

The cassava variety YTP2 (Me 681) developed through selection from Thondamuthur type at Tapioca and Castor Research Station, TNAU, Yethapur. The overall performance of this revealed that the high tuber yield (42.20 t ha⁻¹) and starch content (28.40%) which is 15.94% and 18.20% increase over the check varieties YTP1 and H226 respectively. The results of DNA fingerprint data involving SSR markers (SSRY235, NS169 and NS928) showed that it is genetically distinct from the existing commercial varieties viz., YTP1, H226 and Sree Athulya.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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