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RESEARCH ARTICLE

Genetic variability and inter species relationship between wild and cultivated yams (*Dioscorea* spp.) from Koraput, India based on molecular and morphological markers

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Abstract Wild yams (*Dioscorea* spp.) are important tuber crops used both as vegetable and medicine by the tribal people of Koraput, India. There is deficiency of documented information on genetic structure and diversity of wild yams and its genetic assessment is necessary for crop improvement program. The present study assessed the level of genetic diversity of eight wild and one cultivated yam species of Koraput by using different morphological and molecular markers. Significant variation in different yield and morphological traits was observed among the studied yam species. The major morphological traits such as branch number, stem thickness, tuber depth, tuber length, number of tubers per plant and yield showed high genetic heritability accompanied with high genetic advance and major determinants of phenotypic diversity. Molecular profiling was carried out by taking five simple sequence repeat markers. A total of 10 polymorphic bands with an average of two were detected at the loci of the five markers across the nine yam species. Genetic similarity analysis revealed that some wild yam species such as *D. oppositifolia*, *D. hamiltonii* and *D. pubera* showed higher genetic similarity with cultivated (*D. alata*) species. The knowledge of the extent of genetic variations of wild yam species is important for planning of the genetic conservation and the utilization of this resource especially for genetic improvement.

Keywords Genetic variability · Genetic advance · Genetic diversity · Tuber yield · Wild yam

Introduction

Yams (*Dioscorea* spp.) are important food security crops for millions of small-scale farmers in the tropical and subtropical regions of the world (Mwiringi et al. 2009; Arnau et al. 2017). There are more than 600 species of yams so far reported in the world, of which only ten species are commercially cultivated (Behera et al. 2009). These species are unique for their food, medicinal and economic values due to the presence of different bioactive constituents (Bhandari et al. 2003; Ngo Ngwe et al. 2015). Despite its economic and cultural importance, breeding and selection of yam genotypes with improved traits is currently inhibited by the lack of adequately characterized wild species both at the morphological and molecular level (Asiedu et al. 1998; Arnau et al. 2017). The dearth of knowledge regarding population structure has significantly contributed to genetic erosion of yams (Dansi et al. 1997). Information on genetic diversity and relationships within and among the yam species are essential for their efficient utilization in the crop improvement programs (Mohammadi and Prasanna 2003; Ferguson 2007). Earlier some attempts have been made to use molecular markers for assaying genetic diversity to estimate the relationship among yam accessions and species of Brazil (Siqueira et al.

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2014), Ghanaian species (Mengesha et al. 2013), genotypes from Sierra Leone (Norman et al. 2011), Chinese (Lay et al. 2001) and yams of world-wide (Arnau et al. 2017). However, there is deficiency of documented information on genetic structure and diversity of both cultivated and wild yams of India.

Koraput is one of the tribal dominated districts of Odisha in India (18°14′ to 19°14′N latitude and 82°05′ to 83°25′E longitude) and is recently declared as one of the agro-biodiversity hot spots in India (Mishra and Chaudhury 2012). There are eight wild yam species (*Dioscorea oppositifolia*, *D. wallichii*, *D. hamiltonii*, *D. glabra*, *D. bulbifera*, *D. pubera*, *D. pentaphylla* and *D. hispida*) and one cultivated species (*D. alata),* which have been used as food and medicine by the tribal people of Koraput (Mishra et al. 2011; Padhan and Panda 2016). Recently, their eco-physiological importance and nutritional superiority over other tubers were reported (Padhan and Panda 2018). In spite of their importance as an indigenous food, to the best of our knowledge there is no published study on genetic structure and diversity of wild yam species of India particularly in Koraput. For proper utilization and incorporation of these wild yam species for future crop improvement program, information on genetic structure and diversity is of utmost importance. Further, there is a need to generate information on genetic variability, heritability, and genetic advance of the wild yam species, for better understanding on the variability existing in the population. Therefore, the objectives of the present study were to assess the level of genetic diversity among wild and cultivated yam species of Koraput by using different morphological and molecular markers.

Materials and methods

Plant materials and growth condition

The study was conducted with eight wild edible yam species namely *Dioscorea oppositifolia*, *D. wallichii*, *D. hamiltonii*, *D. bulbifera*, *D. pubera*, *D. glabra*, *D. pentaphylla* and *D. hispida* along with one cultivated species, *D. alata* from Koraput India. The detail of phenotypic characters of different wild and cultivated yam species are presented in Table S1. The plants were grown in the garden of Central University of Orissa, Koraput, India (82°44′54′′E to 18°46′47′′N, 880 m above the mean sea level and average rain fall 1500 mm) during yam growing season (April to December) by following the standard agronomic practices (Padhan and Panda 2018). The experiments were carried out for 2 years in a randomized block design with three replications in each species. The pool data of different yield and morphological parameters of both the year was presented.

Measurement of morphological and yield parameters

Various morphological parameters were measured in flowering stage in three replications as per the standard agro-biometric method proposed by Panse and Sukhatme (1978). Height of the plant and thickness of main stem was measured when all the species attained the maximum linear growth or reached flowering. Number of branches was calculated by counting the branches produced in the main stems. Senescence time was calculated by counting the days from the date of planting to the date when first leaf from the tip turned to yellow. Harvesting of tubers and shoot was done after all the vines dried and it was around 240 days after planting. Number of tubers produced in each plant, length of tuber and width of tuber was also measured. Tuber formation depth was assessed by measuring the soil depth from plain surface of soil to the depth where the tuber was formed.

Measurement of genetic variability

The genetic variability in different yield and morphological parameters among the studied yam species was estimated by computing phenotypic and genotypic coefficients of variation. The genotypic variance (σ^2_G) and phenotypic variance (σ^2_P) was calculated as per Steel et al. (1997). The phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and environmental coefficient of variation (ECV) were calculated via the formula given by Burton and Devane (1953). Broad-sense heritability (h^2_{bs}) and genetic advance, as percentage of mean, were computed via the formula given by Johnson et al. (1955).

Genomic DNA isolation and purification

Total genomic DNA was extracted from the fresh young leaf using standard CTAB (Cetyl trimethyl ammonium bromide) procedure of Doyle and Doyle (1990) with some modification. Leaf material (1–2 g) was ground into powder in presence of liquid nitrogen and homogenized in 10 ml of preheated (65 °C) extraction buffer containing 100 mM Tris HCl pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% CTAB and 0.4% β-mercaptoethanol with insoluble polyvinyl polypyrrolidone (PVPP). Purification of the genomic DNA was carried out by giving RNase A treatment (Mukherjee et al. 2013) followed by phenol: chloroform: isoamyl alcohol (25:24:1) addition and centrifuged at

10,000 rpm for 10 min. The upper phase was collected and equal volume of pre-chilled absolute ethanol was added to precipitate the purified DNA. The DNA was washed with 70% ethanol for 2–3 times and air-dried. The dried DNA was dissolved in 100 µl of TE buffer [Tris 10 mM and EDTA 1 mM (pH 8.0)]. The quality of the DNA was visually assessed by electrophoresis on 1% agarose gel. The DNA concentration was quantified using a spectrophotometer (Biophotometer, Eppendorf, Germany) at 260 nm wavelength.

SSR analysis

Molecular profiling of the studied yam species were carried out by taking five simple sequence repeat (SSR) markers (Arnau et al. 2017). The details of primer and their sequence information were presented in Table S2.The SSR analysis was performed as per the methodology described by Siqueira et al. (2014) with some modifications. Each amplification mixture of 25 µl reaction volume contained: 2.5 µl of 10X PCR assay buffer containing 20 mM $MgCl_2$, 25 mM of dNTPs (dATP, dTTP, dCTP and dGTP), 3U/µl of Taq DNA polymerase (Banglore Genei Pvt. Ltd Bangalore, India), 1 µl of each forward and reverse SSR primer (10 pmol/µl) and 50 ng of template DNA along with 1 µl of 1% BSA and 2.5 µl of 1% PVPP (K-40) to neutralize the phenolic content. The PCR reaction was carried out in thermal cycler (Himedia-Prima, Model 96). The PCR program involved complete denaturation of template DNA at 94 °C for 5 min, followed by 35 cycles at 94 °C for 1 min for denaturation, primer annealing temperature at 46 °C (depending on TM value of primers) for 1 min and polymerization at 72 °C for 1.30 min, with a final extension step at 72 °C for 5 min. After the completion of the PCR reaction, 2.5 µl of 6X DNA loading dye was added to the PCR product. All the PCR reactions were repeated thrice to check the reproducibility.

Polymorphism screening and analysis of amplified products

The amplified products were resolved through agarose gel and documented using a gel documentation system (Alpha Imager, USA). The presence/absence of clearly amplified bands were transformed into a binary character matrix (1 for presence and 0 for absence of a band at a particular position). The genetic similarity index among different *Dioscorea* species were constructed by phylogram using the band scoring data of SSR markers and were measured through paired group (UPGMA) by Jaccard's coefficient of similarity index using NTSYS pc 3.2 software. The primer index was calculated from the sum of polymorphic index (Mukherjee et al. 2013).

Statistical analysis

The statistical significance of the parameter mean was determined by performing the Fisher's test using CROPSTAT (International Rice Research Institute, Philippines) software. The multiple correlation analysis was carried out by Microsoft Excel ver. 2007.

Results and discussion

Variations in yield and morphological traits

Variations in morphological parameters of different yam species from Koraput are presented in Table 1. Significance variation ($P < 0.05$) in plant height was observed among the studied yam species. The range of plant height varied from 3.1 to 4.0 m and it was significantly more in *D. oppositifolia* as compared to the other species. Significant differences ($P < 0.05$) of branch number and stem thickness were observed among the yam species. Branch number and stem thickness ranged from 3.5 to 13.0 and 0.28 to 0.71 cm, respectively. Similarly different tuber characteristics such as tuber depth, tuber length and tuber width varied significantly ($P < 0.05$) among the studied yam species (Table 1). The range of tuber depth, tuber length and tuber width in studied yam species varied from 11.9 to 47.1 cm, 16.1 to 54.2 cm and 3.2 to 17.2 cm respectively (Table 1). Some wild yam species, such as *D. oppositifolia, D. hamiltonii* and *D. pubera* showed remarkably higher tuber length in comparison to cultivated species (*D. alata*). The tuber depth was significantly more in cultivated species. The number of tuber also varied significantly (1.0–5.0) among the studied yam species (Table 1). Some wild species *D. wallichii* and *D. glabra* showed significantly ($P < 0.05$) higher tuber number compared to other species (Table 1). Further, senescence time and shoot and tuber ratio varied significantly i.e. 95 days to 146 days after planting and 1.9–4.0, respectively (Table 1). The senescence time and shoot and tuber ratio was significantly ($P < 0.05$) higher in cultivated yam (*D. alata*) as compared to the wild yams (Table 1). In addition, significant ($P < 0.05$) variation in tuber yield was observed among the wild and cultivated yams and the range of tuber yield was 2.3–3.7 kg $Plant^{-1}$ (Table 1). Some wild yams such as *D. hamiltonii*, *D. pubera*, *D. hispida,* and *D. glabra* showed significantly higher yield compared to the cultivated yam (*D. alata*). Such variation among the yam species might be related to their genetic origin, genetics of the species and geographical sources where they are grown. These results of morphological variations in yam species were also consistent with the previous report of yams of Odisha by Behera et al. (2009), yams of Sierra Leone (Norman et al.

Table 1 Variation in tuber yield and morphological parameters in different yam species from Koraput

Species	Plant height (m)	Branch number	Stem thickness (cm)	Tuber depth (cm)	Tuber length (cm)	Tuber width (cm)	Number of tuber	Shoot: tuber	Yield (Kg)	Senescence time
D. oppositifolia	4.0 ± 0.14^{a}	11.0 ± 0.7^{a}	0.28 ± 0.03^{c}	41.7 ± 1.2^{b}	54.2 ± 1.3^{a}	14.9 ± 0.9^{b}	2.0 ± 0.1^{c}	3.7 ± 0.9^{a}	3.0 ± 0.6^{b}	122.0 ± 2.2^{b}
D. hamiltonii	3.8 ± 0.22^{a}	5.5 ± 0.8^{c}	0.31 ± 0.05^{bc}	41.2 ± 1.0^{b}	50.6 ± 0.9^{a}	14.0 ± 0.8^{b}	2.0 ± 0.2^{c}	2.9 ± 0.7^{b}	3.5 ± 0.4^{a}	117.0 ± 3.1^{b}
D. pubera	3.9 ± 0.25^{a}	8.5 ± 0.5^{b}	0.35 ± 0.02^{b}	42.7 ± 1.3^{b}	53.1 ± 1.1^{a}	14.2 ± 0.7^{b}	2.0 ± 0.1^{c}	3.8 ± 0.6^{a}	3.7 ± 0.3^{a}	136.0 ± 4.1^{b}
D. wallichii	3.3 ± 0.24^{b}	13.0 ± 0.7^{a}	0.44 ± 0.04^{b}	41.2 ± 1.0^{b}	48.4 ± 1.2^{ab}	9.5 ± 0.4^{c}	5.0 ± 0.3^{a}	3.4 ± 0.4^{a}	3.0 ± 0.2^{b}	117.0 ± 3.4^{b}
D. hispida	3.3 ± 0.22^{b}	3.5 ± 0.4^{c}	0.71 ± 0.03^{a}	17.1 ± 0.9^{d}	16.9 ± 1.0^{d}	17.2 ± 0.5^{a}	4.0 ± 0.2^{b}	1.9 ± 0.2^{c}	3.3 ± 0.1^{a}	95.0 ± 4.3^{c}
D. pentaphylla	3.1 ± 0.21^{b}	4.5 ± 0.5^{c}	0.40 ± 0.01^{b}	11.9 ± 0.8^{e}	39.6 ± 0.8^{b}	13.5 ± 0.6^{b}	1.0 ± 0.1^{d}	2.8 ± 0.1^{b}	2.3 ± 0.1^{c}	124.0 ± 5.3^{b}
D.bulbifera	3.7 ± 0.23^{a}	4.5 ± 0.3^{c}	0.70 ± 0.02^{a}	22.8 ± 1.0^{c}	16.1 ± 0.9^{d}	12.0 ± 0.8^{bc}	1.0 ± 0.1^{d}	2.9 ± 0.2^{b}	2.8 ± 0.2^{c}	97.0 ± 3.2^{c}
D. glabra	3.4 ± 0.20^{ab}	8.5 ± 0.2^{b}	0.28 ± 0.03^{c}	36.9 ± 1.2^{b}	49.7 ± 0.8^{a}	3.2 ± 0.9^{d}	4.5 ± 0.2^{a}	3.5 ± 0.3^{a}	3.5 ± 0.3^{a}	117.0 ± 4.6^{b}
D. alata	3.8 ± 0.22^{a}	7.5 ± 0.3^{b}	0.30 ± 0.01^{bc}	47.1 ± 1.0^{a}	44.8 ± 1.0^{b}	15.2 ± 0.8^{b}	1.5 ± 0.1^{cd}	4.0 ± 0.2^{a}	3.0 ± 0.2^{b}	146.0 ± 3.6^{a}
Mean	3.5	7.4	0.4	33.6	41.5	12.6	2.5	3.2	3.1	119.0
LSD ($P < 0.05$)	0.5	2.8	0.1	4.2	5.0	1.4	0.9	0.7	0.4	10.1
CV (%)	7.0	17.8	5.4	5.5	5.2	5.0	4.6	9.9	6.9	3.8

Data are the mean of three replications ± standard deviation (n = 3). Means followed by a common letter in the same column are not significantly different at the 5% level by *Fisher's* least significance difference (LSD) test

Table 2 Genetic variability parameters such as range, mean, standard error (SE), genotypic variation (σ^2_G), phenotypic variation (σ^2_P), environmental coefficient of variation (ECV), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (h^2_{bs}), genetic advance (GA) and genetic advance as percentage of the mean (GAM) for tuber yield and morphological parameters in different yam species from Koraput

Traits	Range	Mean ± SE	σ^2_G	σ^2_P	ECV (%)	GCV (%)	PCV (%)	h^2_{bs} (%)	GA	GAM
Plant height	3.1–4.0	3.49 ± 0.17	0.10	0.13	6.95	9.13	10.36	77.54	0.58	16.65
Branch number	3.5–13.0	7.00 ± 0.88	9.89	10.67	17.82	44.92	46.66	92.71	6.24	89.10
Stem thickness	0.28–0.71	0.41 ± 0.02	0.03	0.03	0.00	42.57	42.57	99.00	0.35	87.70
Tuber depth	11.9–47.1	33.60 ± 1.31	140.24	141.96	5.53	35.24	35.46	98.78	24.25	72.16
Tuber length	16.1–54.2	41.51 ± 1.56	201.18	203.60	5.30	34.18	34.38	98.81	29.04	69.99
Tuber width	3.2–17.2	12.61 ± 0.42	20.40	20.58	4.75	35.85	36.00	99.13	9.26	73.52
Number of tuber	1.0–5.0	2.50 ± 0.28	2.60	2.68	16.06	64.50	65.49	96.99	3.27	98.96
Shoot: tuber	1.9–4.0	3. 24 ± 0.22	0.37	0.42	9.63	18.98	20.16	88.59	1.18	36.79
Yield	2.3–4.7	3.12 ± 0.19	1.24	1.28	8.60	35.93	36.44	97.22	2.26	72.98
Senescence time	92.0–146.0	119.00 ± 3.14	295.07	304.96	3.74	14.43	14.67	96.76	34.81	29.25

Table 3 Relationship between tuber yield and morphological parameters in different yam species

Parameters	Plant height	Branch No	Stem thickness	Tuber depth	Tuber length	Tuber width	Tuber No	Shoot: tuber	Yield	Senescence time
Plant height	1.000									
Branch No	0.225^{ns}	1.000								
Stem thickness	− 0.345^{ns}	− 0.526*	1.000							
Tuber depth	0.542**	0.803**	− 0.627**	1.000						
Tuber length	0.348^{ns}	0.694**	− 0.947**	0.728**	1.000					
Tuber width	0.297^{ns}	− 0.366^{ns}	0.266^{ns}	− 0.261^{ns}	− 0.263^{ns}	1.000				
Tuber No	− 0.303^{ns}	0.339^{ns}	− 0.056^{ns}	0.407^{ns}	0.191^{ns}	− 0.432*	1.000			
Shoot: Tuber	0.555**	0.696**	− 0.746**	0.720**	0.742**	− 0.270^{ns}	− 0.197^{ns}	1.000		
Yield	0.360^{ns}	0.600**	− 0.447*	0.696**	0.528**	− 0.338^{ns}	0.514*	0.528*	1.000	
Senescence time	0.052^{ns}	0.120^{ns}	− 0.531*	0.269^{ns}	0.471*	− 0.056^{ns}	− 0.154^{ns}	0.559**	0.219^{ns}	1.000

Total degrees of freedom = 26,*$P < 0.05$; **$P < 0.01$

ns non significant

2011) and Ethiopia (Beyene 2013). As compared to the cultivated yam species (*D. alata*), very little information is available on the yield potential of wild species. Many wild species could not be domesticated primarily due to their poor yield and tubers of inferior quality. The ability to form tubers is dependent on the genetics of the variety (Martin and Rhodes 1978) and is affected by environmental factors such as day length, temperature and some cultivation practices (King and Risimeri 1992). Apart from the yield of tuber, quality of the tuber also plays an important role for selection and domestication (Padhan and Panda 2018). However, the result of these phenotypic traits of wild yam species may provide baseline information for developing a more efficient agro technology for yam cultivation and selection.

Genetic variability, heritability and genetic advance

The extent of variability with respect to various morphological traits in studied yam species were evaluated in terms of phenotypic variances (σ^2_P), genotypic variances (σ^2_G), phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) (Table 2). The traits showing wide range of variation provide ample scope for efficient selection in crop improvement (Mohapatra et al. 2017). The GCV was less than that of PCV and low

differences was observed between them for all the morphological traits in the studied yam tuber (Table 2), which indicates high contribution of genotypic effect for phenotypic expression of traits and these characters were least influenced by the environment. The results were also consistent with the earlier report in different yam species (Beyene 2013; Alam et al. 2014). According to Deshmukh et al. (1986), PCV and GCV values greater than 20% are regarded as high and values between 10 and 20% as medium, whereas values less than 10% are considered to be low. Based on the results of the present finding, high PCV and GCV values were recorded in the traits such as branch number per plant, stem thickness, tuber depth, tuber length, tuber width, and number of tuber per plant and yield of the tuber (Table 2). This indicates the existence of substantial variability in such characters, based on which selection may be effective. Heritability in broad sense indicates the effectiveness of selection on the basis of phenotypic performance, and does show role of genetic progress for selection, which is possible by using the estimate of genetic advance (Sangaré et al. 2017). Heritability estimates ranged from 77.54% to 99% (Table 2). The heritability of tuber yield was 97.22%, which was more than the reported value of Ethiopian yams (Beyene 2013) and tuber yield of potato (Baye et al. 2005). The characters with maximum heritability and high genetic advance as percentage of mean plays pivotal role in selection, as these characters are controlled by the additive genes and less influenced by environment (Panse and Sukhatme 1995). In the present investigation, high estimates of heritability were observed for stem thickness (99), tuber width (99.13), tuber length (98.81) and tuber depth (98.78) suggesting that selection should be effective for these characters, as high heritability implies low influence of the environment. Heritability estimates (above 60%) along with genetic advance (above 20%) will be beneficial for selection of characters (Singh et al. 2011). The genetic advance as percentage of means (GAM) for studied traits ranged from 16.60% to 98.96% (Table 2). In the present study, high GAM along with high heritability was observed in branch number, stem thickness, tuber depth, tuber length, number of tubers per plant and yield. It indicates that these characters would be useful as a base for selection in yam improvement.

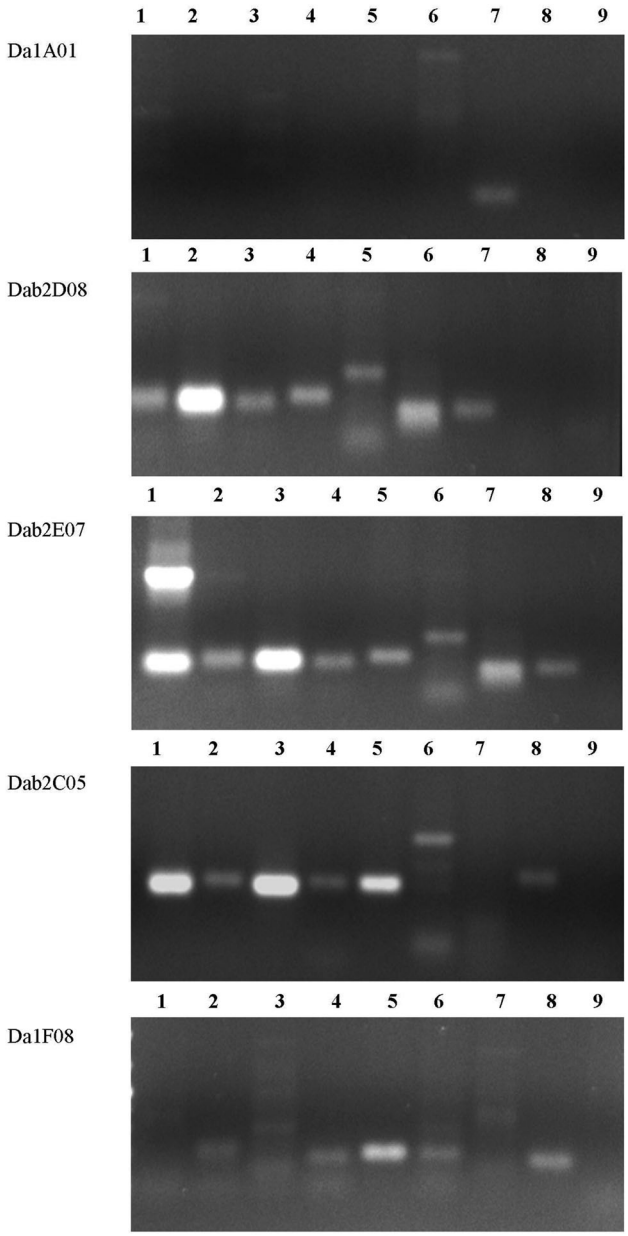

Fig. 1 PCR banding pattern by different markers in wild and cultivated yam species of Koraput

Relationship between the tuber yield and morphological parameters

Relationship between tuber yield and morphological parameters in different yam species were studied by multiple correlation analysis (Table 3). A strong positive correlation ($P < 0.01$) was observed between tuber yield with branch number, tuber depth, tuber length, tuber number and shoot: tuber ratio, whereas it was negatively correlated with stem thickness and tuber width (Table 3). The correlation between tuber yield with plant height and senescence time was not significant. The results indicate that the observed variations in yield of the tuber in different yam species were not based on plant height and stem thickness but related to the branch number. Earlier studies pointed out that the yield is very low in deep seated tuber because species with deepest tubers invariably suffer from moisture stress (Behera et al. 2009). However, in the present study

Table 4 Jaccard's similarity coefficient (below diagonal) and Nei and Lee Dice coefficient (above diagonal) among different wild and cultivated yams based on banding patterns of SSR markers

Species	*D. oppositifolia*	*D. hamiltonii*	*D. alata*	*D. pubera*	*D. wallichii*	*D. pentaphylla*	*D. bulbifera*	*D. glabra*	*D. hispida*
D. oppositifolia	**1.000**	0.432	0.493	0.327	0.443	0.622	0.357	0.451	0.536
D. hamiltonii	0.154	**1.000**	0.500	0.315	0.469	0.680	0.576	0.326	0.571
D. alata	0.325	0.267	**1.000**	0.476	0.730	0.647	0.738	0.471	0.478
D. pubera	0.267	0.356	0.304	**1.000**	0.345	0.586	0.443	0.250	0.537
D. wallichii	0.361	0.178	0.211	0.278	**1.000**	0.481	0.356	0.468	0.611
D. pentaphylla	0.208	0.153	0.109	0.200	0.213	**1.000**	0.750	0.606	0.551
D. bulbifera	0.227	0.137	0.214	0.213	0.270	0.378	**1.000**	0.506	0.695
D. glabra	0.390	0.245	0.200	0.222	0.250	0.327	0.170	**1.000**	0.485
D. hispida	0.100	0.373	0.189	0.344	0.204	0.156	0.231	0.218	**1.000**

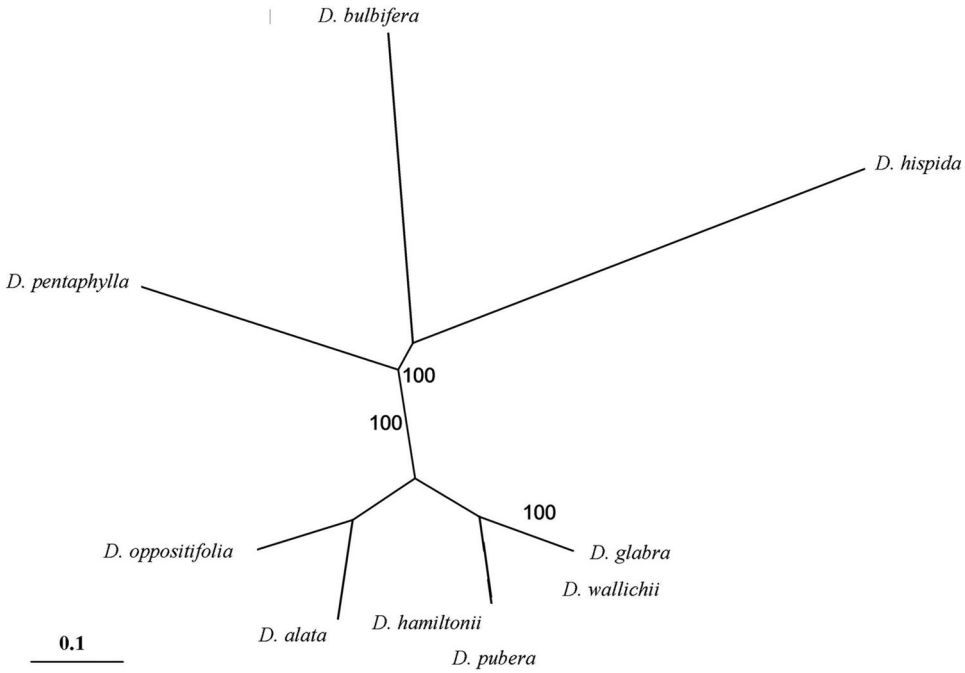

Fig. 2 Rooted phylogram showing the similarity among wild and cultivated yam species of Koraput

high yielding tuber showed the higher tuber depth because of agro-management in the experimental plot. Further, earlier study suggested that yam plant should be of a short duration with high yield (Degras et al. 1977; Oyolu 1982); however, in the present study, we could not find any significant relationship with the tuber yield and senescence time.

Molecular profiling of yam species

Molecular profiling of the studied yam species was carried out by earlier reported SSR markers in different yam species (Arnau et al. 2017). All the primers gave a wide range of amplified fragments ranging from 200 to 1000 bp. Different alleles in the form of variation in molecular weight of each amplified products for each SSR marker are presented in Fig. 1. The markers amplified a total of 10 alleles with an average of 2 alleles per locus (Table S2). The highest number of the allele was obtained with primer Dab2D08 and lowest number of allele was found with primer Da1A01. SSRs markers provide rich genetic information with good genome coverage (Lekha et al. 2010; Turyagyenda et al. 2012). The level of genetic diversity was lower than that of earlier report in different yam species by Arnau et al. (2017). Our results are in agreement with the other reports on genetic diversity among yam species (Bornet et al. 2002; Zhou et al. 2008; Wu et al. 2014). The low level of genetic diversity among yam species might be due to the similar origin, ecotype and speciation as different yam species were collected only

from, Koraput. The results of the present investigation clearly demonstrate the usefulness of these SSR markers to delineate the inter-species relationships among wild and cultivated yam species.

Inter-species relationship among the yam species

The pair-wise genetic similarity is the measure to identify the underlying genetic relationship among the wild and cultivated yam species. The genetic similarity was calculated by Jaccard's similarity coefficient and it ranged from 0.100 to 0.390 among the nine yam species (Table 4). Based on the genetic similarity analysis, some wild yam species such as *D. oppositifolia, D. hamiltonii* and *D. pubera* showed highest genetic similarity with cultivated (*D. alata*) species. Similarly, genetic distance varied from 0.315 to 0.738 among the studied yam species (Table 4). Based on the results, the wild species such as *D. pentaphylla, D. wallichii and D. bulbifera* showed higher Nei and Lee Dice coefficient with cultivated (*D. alata*) species and showed highly genetically distant from cultivated species.

The rooted phylogram was constructed based on the jaccard similarity among the yam species which showed a clear separation of the species into two distinct branches (Fig. 2). The wild species such as *D. oppositifolia*, *D. hamiltonii*, *D. pubera*, *D. wallichii* and *D. glabra* were placed in one branch along with the cultivated species *D. alata*, whereas, *D. pentaphylla*, *D. bulbifera* and *D. hispida* were distributed in another branch and proved their genetic dissimilarity with other yam species.

In conclusion, significant morphological and genetic variability was observed between wild and cultivated yams of Koraput. The major morphological traits such as branch number, stem thickness, tuber depth, tuber length, number of tubers per plant and yield are the major determinants of phenotypic diversity among studied yam species. Based on the genetic similarity analysis, it is revealed that some wild yam species such as *D. oppositifolia, D. hamiltonii* and *D. pubera* showed highest genetic similarity with cultivated (*D. alata*) species and showed their potentiality for yam improvement programs. The information generated in this study will be valuable for breeding and conservation of yam species of Koraput.

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Author contributions BP and DP designed the experiments, cultivated the plants and performed the measurement of morphological traits. BP, AKM and SKM performed the molecular analysis. SKL and DP analyzed the data and wrote the paper. All authors read and provided helpful discussions for the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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