Electronic Journal of Plant Breeding, 5(4): 695-701 (Sep 2014)
ISSN 0975-928X

Research Article

Genetic diversity analysis using shoot and root morphological markers in castor (*Ricinus communis* L.)

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(Received: 01 July 2014; Accepted: 19 Nov 2014)

Abstract

The morphological variation and genetic diversity in 15 root and shoot characters was studied in 27 castor accessions sown in an elevated temporary root study structure. Variation in characters accounted to 3.05 to 50.29%. Characters were subjected to Shannon Weaver diversity index (H`) to know the genetic diversity. Eleven traits recorded high H` indicating suitability in breeding programmes. Their regression coefficients indicated positive change for six traits in dependent variable seed yield. Phenotypic correlation studies revealed that seed yield was significantly correlated to root dry weight, root diameter, plant height, node number, effective spike length and 100 seed weight. Principal component analysis (PCA) revealed that PC1, 2 and 3 accounted for 44.52, 15.93 and 10.54% variation. High loadings in the first three PCs were recorded for nine traits viz., root dry weight, shoot dry weight, root length, total root length, root diameter at crown region, SCMR, effective spike length, node number to primary spike. Hence, present studies gains importance in understanding the root related traits and their role in quantifying the genotypes in terms of divergence.

Key words

Castor, Principal component analysis, Genetic diversity, Phenotypic diversity index, Morphological markers

Introduction

Castor (*Ricinus communis* L.) an important non edible oilseed crop is grown in tropical and semi tropical regions of the world. Tolerance to environmental stress is one of the strengths of the crop (Severino *et al.* 2012). India earns a foreign exchange to a tune of 2253 crores per aaaum from the sale of castor seed (Hegde, 2010). The productivity of the country in 2011-12 is 1417 kg ha^{-1} vis a vis world average of 850 kg ha^{-1} (Anonymous, 2013). Emphasis of current breeding programmes in India is on high seed yield, increased oil content, resistance to *Fusarium* wilt, gray mold, leaf hoppers and capsule borer (Lavanya and Solanki, 2010). Selection of agronomic traits appears to be the key criteria for crop improvement.

Heritability of characters (Solanki and Joshi, 2000), GCA and SCA for seed yield, seed yield components and other agronomic traits reveal that selection in a conventional breeding could enhance these traits (Ramesh *et al.,* 2005: Nobrega *et al.,* 2010). In all these studies, above ground characters were only considered and they contributed to increased biomasss. However, root system architechture (RSA) traits are also known to impart drought stress tolerance in crops and variation in root traits was observed to be large (Udaya Kumar, 2002).

Root studies in castor include, correlation of length and weight of roots (Smith *et al.,* 1991), root and shoot interrelationship (Sarada *et al.,* 2010), water use efficient lines showing better root characters (Lakshamamma *et al.,* 2010) and varieties with good root growth showing less seed yield reduction (Lakshamamma *et al.,* 2012). Very few divergence studies were carried out in castor based on root related characteristics. Realizing the importance of shoot and root characters, the present study was taken up to explore the variation, correlation and contribution in morphological traits.

Materials and Methods

Twenty seven germplasm accessions were obtained from Regional Agricultural Research Station, Palem, Acharya N. G. Ranga Agricultural University, and Directorate of Oilseeds Research, Rajendranagar. The accessions represented elite lines (16), pistillate lines (2), male lines (2), wilt tolerant lines (2), early flowering, resistant to reniform nematode and leaf hopper (1) and four checks (Kranthi, Haritha, Aruna, and Kiran). The accessions were planted in Randomized Block Design in a specially designed temporary root study structure 25 m length, 4m width and 1.5 m height with a permanent wall separating the replications to enable root studies. Crop was raised with two replications with spacing of 90 x 45 cm during late *Rabi* 2011 and 2012. Observations were recorded on four plants in each replication for 15 quantitative traits. Data were recorded on standing crop with respect to SPAD chlorophyll meter readings (SCMR) in the morrning hours (8.00 to 9.30 hours) with the help of hand held Minolta SPAD chlorophyll meter (Minolta Corp., Ramsey, New Jersey, USA), relative water content (RWC) using the formula (fresh weight – dry weight / turgid weight – dry weight) x 100, PS II efficiency (Fv/Fm), plant height up to primary raceme, number of nodes up to primary raceme, effective length of main spike, 100-seed weight and seed yield. Carbon Isotope Discrimination (CID expressed in per mill (%o) was done at National Facility for Stable Isotopes, University of Agricultural Sciences, by feeding leaf samples in Infra Red Mass Spectrometry (IRMS) facility in Bangalore. Structure was dismantled 110 DAS when root growth was maximum and data recorded on RSA traits viz., root diameter at crown region, root length, number of laterals, total root length, root dry weight and total dry weight. ANOVA, Principal Component Analysis (PCA) was carried out to study the variation and extent of relationship between yield and the morphological and root characters. The diversity index (H`) of Shannon and Weaver diversity index (1949) was used as a measure of the phenotypic diversity of each trait.

Results and discussion

The knowledge of morphological traits and their adaptability to specific microclimates enables development of new cultivars with traits of interest. Remarkable phenotypic variation has been observed in species of castor. The mean, minimum and maximum values have been presented for the 15 quantitative characters, their standard deviation and coefficient of variability (Table 1). Variance, a measure of variability is defined as the average of the squared deviation from the mean. In the present study, variation in characters accounted to range from 3.62% to 50.29%. High variance was recorded for shoot dry weight (45.12%), root dry weight (50.29%), number of lateral roots (44.25%) and seed yield (38.57%). Moderate variance was observed for plant height (25.09%), effective spike length (28.84%), root length (29.6%) and total root length (25.76%). Variation important to breeding objectives has been previously reported in castor for phenotypic characters (Fernández-Martinez and Velasco, 2012), oil and proteins (Morris *et al.*, 2011), flowering expression (Lavanya and Gopinath, 2008), RSA traits (Lakshamamma *et al.*, 2010) and molecular markers to access genetic diversity (Vasconcelos *et al.*, 2012).

The phenotypic variation observed in castor does not seem to reflect the high levels of genetic diversity. Germplasm collections worldwide have shown low levels of variability and lack of geographically structured genetic populations regardless of the marker system used (Qui *et al.*, 2010). Little is known about the actual genetic diversity of the species despite the publication of castor genome (Chan *et al.*, 2010) and very few studies have been carried out to to access genetic diversity in castor. Shannon and Weaver diversity index was calculated to compare the phenotypic diversity index (H`) for morphological characters. The index is generally used in genetic studies to measure both allelic richness and evenness. However because of log transformation it is not readily interpretable in genetic terms. A low H` indicates an extremely unbalanced frequency class for an individual trait and a lack of genetic diversity. H` estimates for 15 traits showed significant variation (Table 2). H` ranged from 0.446 to 0.586. High values for H' were observed for traits like CID, root diameter at crown region, Fv/Fm ratio, SCMR, total root length, effective spike length, shoot dry weight and root dry weight. H` values were low for plant height, number of laterals and node number. Plant breeding involves mostly traits that are associated with economic gain. Such of these traits require constant genetic enhancement and in turn appropriate quantitative analysis. Also it is understood that genetic component of variance is trait specific. Hence, to include total genetic information, as many important traits as possible have to be included in any divergence analysis (Arunachalam, 2004). Also very few divergence studies were carried out in castor based on root related characteristics which has direct relevance with the physiological studies for the identification of the water use efficient genotype. Hence, present studies gains importance in understanding the root related traits and their role in quantifying the genotypes in terms of divergence.

Regression coefficient (b) revealed that ten plant traits contributed to seed yield (Table 3). Step down multiple regression gave values of R^2 and R^2 adjusted as 0.9227 and 0.8744. Six characters accounted for positive change while four characters accounted for negative change in the dependent variable (Table 3).

Phenotypic correlation studies were carried out to understand the relationship among plant characters with seed yield (Table 4). Correlation values >0.71 have been suggested to be meaningful, wherein > 50% of the variation in one trait is predicted by the other. Shoot dry weight was significantly correlated with root dry weight and root diameter. Root dry weight showed positive significant correlation with root diameter, tap root length and total root length. Root growth was correlated to RWC. Genotypes with better root characters showed positive correlation with TDM and in turn were considered as best lines for WUE (Lakshamamma *et al.*, 2010). RSA traits like root diameter showed significant correlation with tap root length and total root length. Root dry weight, root diameter and tap root length significantly correlated with seed yield a function of water transpired, WUE and HI (Passioura, 1986). These results strongly show the dependence of plant growth on biomass and root traits. However it is difficult to measure transpiration and / or root biomass. To address the issues of biomass above and below ground isotope studies are crucial. Transpiration efficiency (TE) which addresses WUE could be estimated by measuring CID (Δ^{13}C) in leaves (Farquhar *et al.*, 1982). Existence of genetic differences for TE has been reported (Wright *et al.,* 1994). Enrichment of oxygen isotopes (Δ^{13}O) along with leaf area can be used as a rapid and accurate approach to estimate the root biomass.

The above ground characters influenced crop growth, root growth and seed yield. Dependent character like seed yield was significantly correlated to root dry weight (0.5585), root diameter (0.5842), plant height (0.5476), node number (0.7641), effective spike length (0.6868) and 100 seed weight (0.6064) (Table 4). The Δ^{13}C isotope is hypothesized to be associated with higher mean transpiration rate and stomatal conductance (Udayakumar *et al.,* 1998). Selection for low Δ^{18}O that quotes for high TE can result in production of more dry matter (Chunnilal *et al.,* 2005). From these studies it is understood that inclusion of many traits in a breeding programme can enhance the genetic variance and hasten castor improvement.

PCA facilitates selection of genotypes especially when many traits are involved. Fifteen agromorphological traits that contributed to high levels of variability to total variation were subjected to PCA. Their per cent variation for the first three PCs and the vector loadings for each character have been presented (Table 5). The first three PCs had eigen values > 1 i.e., 6.69, 2.39 and 1.58. These three PCs explained 70.99% of variation in castor accessions. In other studies 24 morphological characters were evaluated wherein first five PCs accounted for 93.9% (Bolaji, *et al.*, 2013) and studies on 32 traits, first six PCs accounted to 93% of the total variation (Goodarzi *et al.,* 2011). PC1 which is the first and most important component accounted for 44.52% variation followed by PC2 with 15.93% and PC3 with 10.54%. The traits viz., shoot dry weight, root dry weight, root diameter, root length and total root length had positive loadings to PC1. SCMR, CID and effective spike length showed more weight to PC2, while RWC, fluorescence, and number of lateral roots had negative loadings. These results in castor indicate that genotypes with high PC values have a low RWC, Fv/Fm and number of laterals. Castor crop chiefly grown as a dry land crop cannot afford to have more lateral spread but could have improved RSA traits only. PC3 had high loadings from plant height, CID and number of laterals. Negative loadings were recorded from RWC and shoot weight. It is important to note that to study the variation and extent of relationship between yield and the morphological and root characters in castor, PCA is important tool and can be used effectively even if the numbers of genotypes are limited.

Accessions that show high values for various characters like shoot dry weight (> 80g), root dry weight (≥ 30g), RWC (> 80%), SCMR (> 50), root diameter (> 8cm), number of lateral roots (40), root length (> 180cm) and total root length (>1000cm). At least seven accessions have > 2 common superior traits. Five lines recorded both high shoot and root traits and included Kranthi, Haritha, RG 48, PCS 252 and SKI 215 (Table 6).

Based on the analysis of the data involving the castor accessions it can be concluded that nine traits viz., root dry weight, shoot dry weight, root length, total root length, root diameter at crown region, SCMR, effective spike length, node number to primary spike had high weight in the first three PCs indicating their importance for selection in castor improvement.

Acknowledgements
Authors sincerely acknowledge the financial support provided under the UGC, New Delhi Major Research Project grant No.40-40/2011 (SR) to carry out the present work.

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Table 1. Mean, range and CV of 15 quantitative characters of 21 castor genotypes

Characters	Mean	Minimum	Maximumh	CV (%)	Probability
Shoot dry weight (g)	56.66	19.3	108.1	45.12	**
Root dry weight (g)	18.3	3	38.9	50.29	**
Relative water content (%)	76.24	67.3	83.3	5.33	**
SCMR	49.73	45.9	53.9	3.62	*
Fv/Fm ratio	0.59	0.5	0.68	8.42	**
Plant height (cm)	49.63	33.5	85.5	25.09	**
Node number	10.01	8	14	12.73	**
Effective spike length (cm)	19.69	10.4	30.3	28.84	**
100 seed weight (g)	22.5	17.1	28.6	15.51	**
CID (%o)	18.56	17.6	19.4	3.05	**
Root diameter (cm)	6.58	3.68	8.75	23.75	**
Number of laterals	35.47	16.3	74.8	44.25	**
Root length (cm)	128.29	74.5	225.3	29.6	**
Total root length (cm)	906	503.1	1341.3	25.76	**
Seed yield (kg $ha^{-1)}$	814.3	459.6	1621.3	38.57	**

Table 2. Shannon Weaver index for 15 characters of castor

Plant traits	H` index
Shoot dry weight (g)	0.547
Root dry weight (g)	0.544
Relative water content (%)	0.524
SCMR	0.570
Fv/Fm ratio	0.580
Plant height (cm)	0.497
Node number	0.443
Effective spike length (cm)	0.556
100 seed weight (g)	0.539
CID (%o)	0.586
Root diameter (cm)	0.584
Number of laterals	0.494
Root length (cm)	0.525
Total root length (cm)	0.568
Seed yield (kg ha^{-1})	0.446
mean	0.5334
SE±	0.0119

Table 3. Regression coefficients of 10 plant characters in 27 castor accessions

Character	Regression coefficient	t probability
INTERCEPT a	-1387.92	0.309
Shoot dry weight (g)	-11.8865	0.000 ***
Root dry weight (g)	20.48222	0.002 **
RWC (%)	44.47032	0.000 ***
Plant height (cm)	13.14199	0.001 ***
Node number	89.71164	0.005 **
Effective spike length (cm)	30.50555	0.003 **
CID (%o)	-154.508	0.011 *
Root diameter (cm)	112.6274	0.008 **
Root length (cm)	-2.04173	0.054
Total root length (cm)	-0.72114	0.003 **

R^2adj=0.8744

Table 4. Correlation among 15 characters in castor accessions

	Root dry weight	Relative water content	SCMR	Fv/Fm ratio	Plant height	Node number	Effective spike length	100 seed weight	CID	Root diameter	Number of laterals	Root length	Total root length	Seed yield
Shoot dry weight (g)	0.84	0.53	0.40	0.47	0.34	0.45	0.51	0.36	0.07	0.76	0.23	0.48	0.47	0.50
Root dry weight (g)	1.00	0.46	0.20	0.49	0.30	0.51	0.42	0.30	0.14	0.71	0.41	0.54	0.54	0.56
RWC (%)		1.00	0.13	0.87	-0.02	0.35	0.21	0.37	-0.20	0.49	0.18	0.54	0.56	0.47
SCMR			1.00	0.06	0.44	0.27	0.68	0.45	0.17	0.24	-0.38	0.18	0.25	0.43
Fv/Fm ratio				1.00	0.00	0.30	0.11	0.37	-0.18	0.43	0.29	0.53	0.50	0.44
Plant height (cm)					1.00	0.24	0.55	0.27	0.35	0.54	0.25	0.53	0.53	0.55
Node number						1.00	0.65	0.52	0.27	0.43	0.15	0.45	0.40	0.76
Effective spike length (cm)							1.00	0.54	0.56	0.39	-0.14	0.44	0.41	0.69
100 seed weight								1.00	0.16	0.44	-0.20	0.51	0.33	0.61
CID (%o)									1.00	0.09	0.01	0.16	0.12	0.16
Root diameter (cm)										1.00	0.39	0.67	0.77	0.58
Number of laterals											1.00	0.36	0.44	0.18
Root length (cm)												1.00	0.68	0.56
Total root length (cm)													1.00	0.54

Note: 0.38 and 0.49 are critical value for 5 and 1 per cent respectively

Table 5. Vector loadings and percentage of variation explained by first three Principal Components in castor

	PC1	PC2	PC3
Eigen value	6.68	2.39	1.58
% var explained	43.07	16.89	11.25
Cum var explained	43.07	59.95	71.2
Shoot dry weight (g)	0.3264	-0.0453	-0.0497
Root dry weight (g)	0.3219	-0.117	0.0913
Relative water content (%)	0.2642	-0.3157	-0.3537
SCMR	0.1811	0.4034	-0.2698
Fv/Fm ratio	0.251	-0.3559	-0.298
Plant height (cm)	0.2307	0.2497	0.3844
Node number	0.2716	0.1271	-0.0756
Effective spike length (cm)	0.2772	0.4197	-0.0433
100 seed weight (g)	0.2491	0.1889	-0.3427
CID (%o)	0.0939	0.3863	0.3135
Root diameter (cm)	0.3466	-0.0919	0.1483
Number of laterals	0.132	-0.3597	0.5234
Root length (cm)	0.3267	-0.0757	0.0903
Total root length (cm)	0.3245	-0.1102	0.1654

Table 6. Castor accessions with better shoot and root characters

Characters	Critical value	Genotypes
High shoot dry weight	> 80g	Haritha, RG 48, RG 47, RG 67, PCS 252
High root dry weight	≥ 30g	Kranthi, Haritha, RG 48, PCS 252
High RWC	> 80%	Kranthi, Haritha, RG 48, RG 43
High SCMR	>50	Kranthi, Haritha, RG 48, RG 47, RG 67, PCS 230 SKI 215, PCS 171, PCS 265, DCS 78, PCS 302
High root diameter	> 8cm	Kranthi, Haritha, PCS 236, PCS 252, SKI 215, PCS 171
More root laterals	> 40	PCS 293, RG 1354, PCS 312, RG 1, PCS 236, PCS 252, SKI 215
High root length	> 180cm	Kranthi, PCS 106, SKI 215
High total root length	> 1000cm	Kranthi, Haritha, RG 48, RG 43, PCS 312, PCS 252, RG 1686 SKI 215, PCS 171