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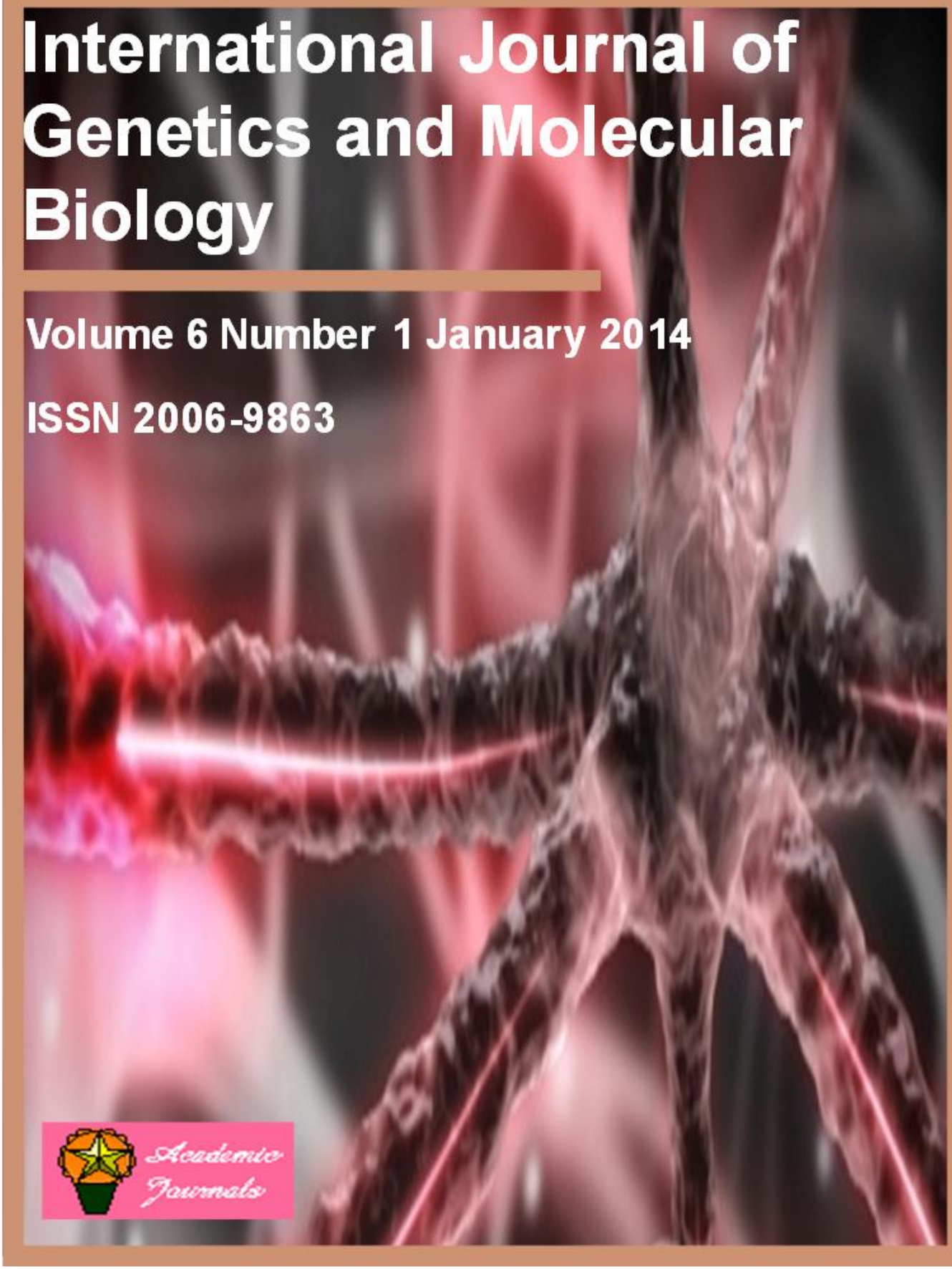

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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Full Length Research Paper

Partitioning and distribution of random amplified polymorphic DNA (RAPD) variation among eggplant *Solanum* L. in Southwest Nigeria

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***Solanum* L., the largest genus of the Solanaceae family, vary morphologically, is diverse in number and is ecogeographically distributed. In Nigeria, previous studies had focused mainly on chromosome morphology, genome description and medicinal values, which are insufficient for genetic affinities. This study used four highly polymorphic random amplified polymorphic DNA primers to describe both the genetic relatedness and variability among 25 accessions of eggplant from Southwestern Nigeria. At a truncated line of 65%, five clusters and two ungrouped samples are distinguishable from the dendrogram. The data reveals that *Solanum dasyphyllum* Schum. & Thonn. is more closely related to *Solanum macrocarpon* L. than to *Solanum melongena* L. The relatedness between *Solanum incanum* L. and *Solanum melongena*, a probability of being progenitors from a common ancestral lineage was also shown. Occurrence of *Solanum scabrum* L. and *Solanum nigrum* L. in the same clusters different from *S. melongena*, is an indication of distant relatedness to *S. melongena* but close relatedness between them. High level of polymorphism was observed in this study going by the coefficient of variation which exhibited a good separation from a conserved region of the genome. This study, therefore, reveals a wide and diverse genetic base in Nigerian eggplant *Solanum*.**

Key words: Eggplant, genome, synonymy, polymorphism, phylogenetic.

INTRODUCTION

Solanum L., a complex and large genus of the family Solanaceae has an unresolved proper delineation of the species. The genus contains roughly between 1,500 and 2,000 species (Bohs, 2001). They are morphologically varied, numerically diversed and vastly ecogeographically distributed. Several species of vegetable *Solanum* important for human diet and health are referred to as eggplant (Daunay et al., 2001). Examples include *Solanum melongena, Solanum aethiopicum, Solanum macrocarpon, Solanum quitoense* Lam., *Solanum sessiliflorum* Dunal and related species. The taxonomy of eggplant *Solanum* has remained challenging due to species' large size, overlapping ecogeographical distribution (Levin et al., 2005), morphological plasticity, similar genomes (Okoli, 1988) and existence of swamps of natural hybrids (Obute et al., 2006; Oyelana and Ugborogho, 2008). The inconsistencies and misconceptions generated by these factors have made past attempts at taxonomically resolving the complexities associated with the genus difficult. The taxonomic

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uncertainties still persist in this genus largely because previous studies to address the taxonomic problem of vegetable *Solanum* have focused mainly on morphology (Karihaloo and Rai, 1995; Kumar et al., 2013), crossability and F1 fertility (Baksh, 1979; Hassan and Lester, 1990a; Lester and Hassan, 1991; Furini and Wunder, 2004) and anatomy (Hassan and Lester, 1990b). Establishing genetic affinities on such parameters are insufficient, as *Solanum* makes successful crosses with putative progenitors as well as distantly related species.

The advent of molecular biology has revolutionized the field of plant systematics and has been used successfully in phylogenetic relationships at all taxonomic levels (Bohs, 2005) as well as in DNA fingerprinting of plant genomes (Cervera et al., 1998) and in genetic diversity studies (Isshiki et al., 2008; Fory et al., 2010). The use of molecular techniques in genetic diversity studies is supported by the finding that evolutionary forces such as natural selection and genetic drift produce divergent phylogenetic branching which can be recognized because the molecular sequences, on which they are based, share a common ancestor (Singh et al., 2006). Random amplified polymorphic DNA (RAPD), when compared with other molecular markers, is more effective in this regard as it is simple, rapid, requires only a small quantity of DNA and it is well adapted for nonradioactive DNA fingerprinting of genotypes (Cao et al., 1999). It is also able to generate numerous polymorphisms (Williams et al., 1990). Karihaloo et al. (1995) focused directly on nuclear genomic diversity of *Solanum* by undertaking RAPD analysis. Karihaloo and Gottlieb (1995) also reported that greater DNA polymorphism exists in weedy *Solanum insanum* than in advanced cultivars of eggplants. RAPD data were used in several other studies such as Miller and Spooner (1999), Stedje and Bukenya-Ziraba (2003) and Singh et al. (2006) to clarify phylogenetic relationships. Other molecular markers have also been previously used to study the variability as well as relatedness among eggplant *Solanum* species. For instance, Nunome et al. (2003a) and Ge et al. (2013) both employed microsatellite markers or simple sequence repeat (SSR) markers, Behera et al. (2006) used STMS markers, Fory et al. (2010) worked on Colombian collection of *Solanum* using amplified fragment length polymorphism (AFLP), and more recently, Ali et al. (2013) studied the diversity among samples of Chinese *Solanum* by comparing results of RAPD and SSR markers.

In Nigeria, not many works have been done on the nature of genetic diversity and characterization of vegetable *Solanum*, especially using molecular methods. Many vegetable *Solanum* species that occur in Nigeria are sources of food and of medicinal importance (Gbile and Adesina, 1988). Taxonomic studies on the vegetable *Solanum* species in Nigeria have been based on chromosome morphology (Oyelana and Ugborogho, 2008), genome description (Okoli, 1988), medicinal and food values (Gbile and Adesina, 1988). These have not resolved the problems of synonymy and taxa misidentification common to the genus. As a result, this study attempts to resolve to a larger extent the taxonomic difficulties associated with vegetable *Solanum* especially among the species found in Southwestern Nigeria using RAPD molecular marker.

MATERIALS AND METHODS

Sample collection and identification

Fresh leaves (young and matured), fruits and seeds of eggplant *Solanum* samples of different species were collected from different locations in Southwestern Nigeria (Longitude 3° 20′E - 5° 10′E and Latitude 6° 15′N - 9° 00′N) especially in areas known for eggplant diversity. Each sample was labelled accordingly. The fresh leaves were prepared for molecular analysis while mature leaves were prepared for herbarium. A total of 25 samples were collected and analyzed in this study. Their authenticated names and places of collection are shown in Table 1. The breakdown showed that the collections consists of 10 different *Solanum* species made of 2 samples of *Solanum dasyphyllum*, 2 of *Solanum nigrum*, 3 *Solanum macrocarpon*, 2 *Solanum torvum*, 1 *Solanum erianthum*, 3 *Solanum melongena*, 7 *Solanum gilo*, 2 *Solanum scabrum*, 2 *Solanum aethiopicum* and 1 of *Solanum incanum*. Figure 1 shows some of the samples with variations in shapes and colours.

Voucher specimens were prepared from the samples following the method of Ogundipe et al. (2009) and sent to Forestry Herbarium Ibadan (FHI) where they were authenticated by taxonomists. These specimens were then deposited at both the University of Lagos Herbarium (LUH) and Forestry Herbarium Ibadan (FHI) for reference purposes.

Total genomic DNA extraction

Total genomic DNA extraction was carried out on young fresh leaves of each sample (Dellaporta et al., 1983). This was followed by additional purification in a silica-column inserted into vacuum manifold connected to a vacuum pump using QIAquick purification kit (Promega). Verification of the quality of the purified DNA samples was achieved by electrophoresis on a 1% Agarose gel.

Polymerase chain reaction (PCR)

Twenty seven (27) Operon primers (Operon Technologies Inc., USA) were screened based on higher GC content (between 60 - 70%) and their previous workability. Only four (4) that are highly polymorphic and gave reproducible bands were selected and used in the analysis of all the 25 genotypes. Total reaction volume for PCR was 10 µl containing 1.0 µl of 10x TAE buffer, 2 µl of 10 mg/µl sample DNA, 1.0 µl $MgCl_2$, 0.8 µl mixture of 10 mM dNTP, 20 (5% Tween), 20 polyoxyethylene sorbitan monolaurate with 20 ethylene oxide units, 4.6 µl of distilled water, and 5 U Taq DNA polymerase (1 U final conc.). Amplification was accomplished on the Techne TC- 412 thermal cycler (Model FTC41H2D, Barloworld Scientific Ltd, Staffordshire, UK), using the following temperature profile: Initial strand separation step of 3 min at 94°C followed by 40 cycles each consisting of a denaturing step of 20 s at 94°C, annealing step of 40 s at 35°C and an extension step of 1 min at 72°C. The last cycle was followed by 5 min extension at 72°C to allow complete extension of the PCR products with a final hold at 4°C till electrophoresis. The reaction was repeated two times for each

Table 1. Eggplant *Solanum* samples and places of collection.

Sample I.D no.	Identification Name	Place of collection	State of collection
OG02	*Solanum dasyphyllum*	Wasinmi	Ogun
OG03	*S. nigrum*	Wasinmi	Ogun
OG04	*S. dasyphyllum*	Joga orile	Ogun
OG05	*S. nigrum*	Joga orile	Ogun
OG06	*S. macrocarpon* (White fruit)	Abulemaria	Ogun
OG07	*S. macrocarpon* (Green fruit)	Abulemaria	Ogun
OG08	*S. torvum*	Wasimi-Imasai	Ogun
OG09	*S. erianthum*	Wasimi-Imasai	Ogun
OG10	*S. melongena* (Green fruit)	Wasinmi-Imasai	Ogun
OY11	*S. gilo* Raddi (White fruit)	Igboho	Oyo
OY12	*S. gilo* Raddi (White fruit)	Igboho	Oyo
OY13	*S. gilo* Raddi (White fruit)	Igboho	Oyo
OY14	*S. gilo* Raddi (White fruit)	Igboho	Oyo
OY15	*S. incanum* L. (Green small fruit)	Igboho	Oyo
OY16	*S. scabrum*	Igboho	Oyo
OY17	*S. aethiopicum*	Igboho	Oyo
OY18	*S. scabrum*	Igboho	Oyo
OY19	*S. melongena* (White fruit)	Igbope	Oyo
OY20	*S. aethiopicum*	Igboho	Oyo
OS21	*S. torvum*	Iwo	Osun
OG22	*S. melongena* (Green fruit)	J3 Camp, Ijebu Ode	Ogun
LA23	*S. gilo* Raddi (Green egg-shaped fruit)	Bariga, Lagos	Lagos
LA24	*S. gilo* Raddi (Green round fruit)	Agbowa-Ikosi	Lagos
LA25	*S. gilo* Raddi (Green round fruit with greenish purple stem)	Agbowa-Ikosi	Lagos
LA26	*S. macrocarpon* (Green fruit)	Agbowa-Ikosi	Lagos

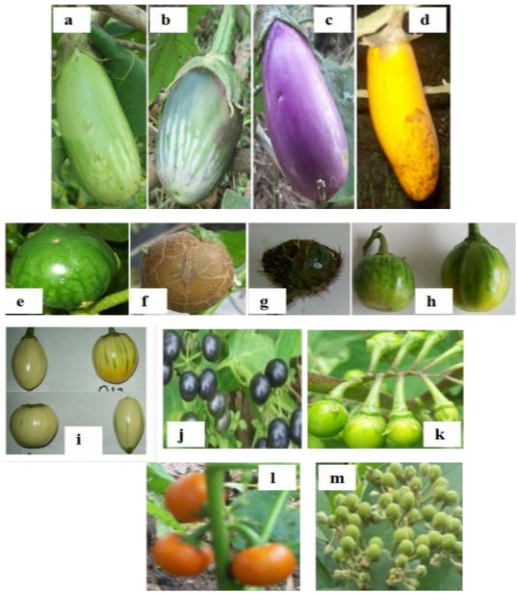

Figure 1. Variability in fruit colour and shape of some eggplant samples studied. Legend: (a - d) *S. melongena*; (e and f) *S. macrocarpon*; (g) *S. dasyphyllum*; (h and i) *S. gilo*; (j) *S. scabrum*; (k) *S. incanum*; (l) *S. aethiopicum*; (m) *S. erianthum.*

Table 2. Operon primers selected with their nucleotide sequence and their characteristic number of bands at amplification in samples analyzed.

Primer used	Primer sequence (5'- 3')	Total number of bands	Number of polymorphic bands	Percentage polymorphic bands (%)
V-19	(5'- GGGTGTGCAG -3')	12	10	83.3
B-18	(5'- CCACAGCAGT -3')	12	10	83.3
OPU-13	(5'- GGCTGGTTCC -3')	13	9	69.2
OPU-15	(5'- ACGGGCCAGT -3')	15	13	86.7
Total		52	42	80.8
Average		13	10.5	

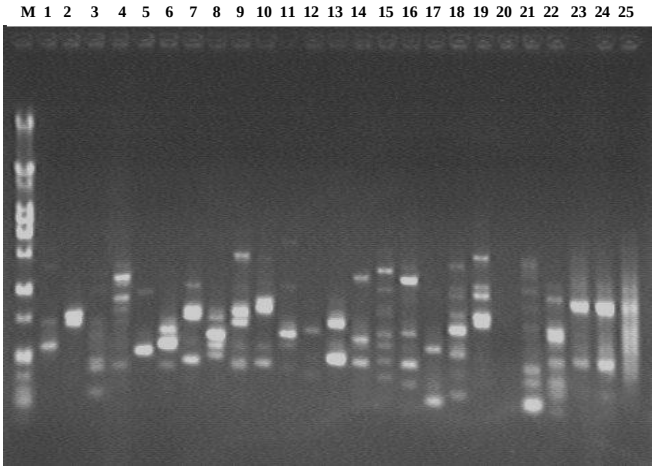

Figure 2. RAPD profiles generated by primer B-18 for *Solanum* samples studied. Legend: M represents the 100 bp DNA Ladder which serves as the reference point; 1 to 25 corresponds to bands produced by the amplified DNA from the 25 samples.

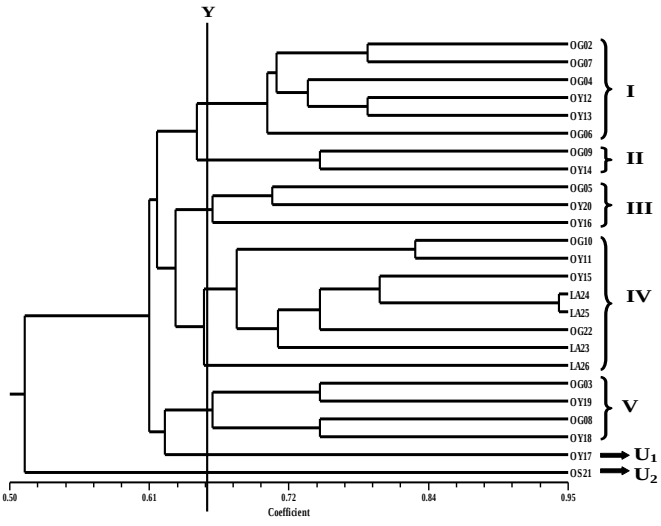

Figure 3. A UPGMA dendrogram showing genetic relationship among accessions of eggplants studied. Legend: Y represents truncated line at a co-efficient of similarity 0.65; I to V represent the five clusters that were distinguishable from the dendrogram while U_1 and U_2 represent ungrouped samples at that co-efficient of similarity.

selected primer to make the result more reliable. 5 µl of each of PCR product (amplicon) were mixed with 3 µl of 10X loading dye (0.25% bromophenol blue, 0.25% xylene cyanol and 40% sucrose, w/v) and spun briefly in a micro centrifuge before loading on a1.5% agarose gel which has been previously stained with safe view. This was run for 1 h 30 min at 110 V/cm. Thereafter, the gel was viewed (with the aid of eye protector) and photographed in the Gel Documentation and Analysis Systems (UVdoc, GA-9000/9010 Version 12).

Data analysis

For each sample, only distinct, well-resolved and unambiguous bands were scored. Faint bands were discarded. The amplified fragments were scored as 1 (present) and 0 (absent) to generate binary matrices. From this matrix, similarity matrices were computed using Sequential Hierarchical and Nested (SAHN) clustering option of the NTSYS-pc 2.02j software package (Rohlf, 1996). The software generated a dendrogram, which grouped the test lines using Unweighted Pair Group Method with Mathematic Average (UPGMA) on the basis of genetic similarity and Jaccard's coefficient.

RESULTS

The RAPD analysis of the 25 samples revealed a total of fifty two (52) bands, amplified by four (4) different oligonucleotide primers namely OPU-13, OPU-15, B-18 and V-19 (Table 2). Forty two (42) of these bands were highly polymorphic with percentage polymorphism put at 80.8% (Table 2). The numbers of amplification products obtained were in the range 12-15. Primers V-19 and B-18 produced the minimum number of (12) bands each, OPU-13 produced 13 bands and primer OPU-15 produced the maximum number of (15) bands. Average of 13 bands was also obtained per primer as shown in Table 2. Figure 2 shows the RAPD profile produced by B-18 Operon primer for the 25 samples.

Jaccard's similarity coefficient matrix generated a dendrogram (Figure 3) based on polymorphism obtained with all the selected four primers using UPGMA clustering option of NTSYS-pc 2.02j software package (Rohlf, 1996). The scale of the dendrogram constructed from the data was between 0.50 and 0.95 with a mean value of 0.73 (Figure 3). At a truncated line of 65% (a similarity co-efficient of 0.65), five clusters (I - V) and two ungrouped

samples (U_1 and U_2) are distinguishable from the dendrogram. Cluster IV is the largest consisting of 8 samples while Cluster II being the smallest is made up of 2 samples (Figure 3). All the samples of *S. dasyphyllum* occur in Cluster I together with 2 samples (out of 3) of *S. macrocarpon* and 2 of *S. gilo*. Cluster IV contains most samples of *S. gilo* together with 2 of *S. melongena* and 1 of *S. incanum* and *S. macrocarpon*, respectively. One sample each of *S*. *nigrum*, *S. aethiopicum* and *S. scabrum* grouped together in Cluster III; so also, Cluster V contains one sample each of *S. melongena*, *S. nigrum*, *S. torvum* and *S. scabrum,* respectively. The only sample of *S. erianthum* occurs with one *S. gilo* in Cluster II while the remaining samples of *S. aethiopicum* and *S. torvum* remained ungrouped U_1 and U_2 respectively. It is worthy of notice that just as the selected primers were able to detect inter-specific polymorphism, they equally did so intra-specifically. This accounted for the occurrence of samples of the same species in different clusters e.g. one sample each of *S. nigrum* and *S. scabrum* occurring in both clusters III and V.

DISCUSSION

Hammond (1979) stated that biosystematics and evolutionary studies have for long time, and for the most part, considered the morphological features of the mature organism. This observation is evident in earlier works on *Solanum* taxonomy such as that of Isshiki et al. (2008), Karihaloo and Rai (1995), Karihaloo and Gottlieb (1995) and Oyelana and Ugborogho (2008). Unfortunately, these and many other studies based on morphological features have not totally resolved the difficulties associated with *Solanum* taxonomy. Discontinuous markers such as random fragment length polymorphism (RFLP), RAPD, AFLP and Single Nucleotide Polymorphism (SNP) have been useful in providing a measure of genetic distances to establish both the taxonomy and phylogenetic relationships among *Solanum* taxa (Karihaloo et al., 1995; Rodriguez et al., 1999; Poczai et al., 2008; Polignano et al., 2009).

The dendrogram constructed based on RAPD data obtained from all the four primers used reflected the morphological variation observed on the samples of eggplant and related species during their collections. It is evident from the dendrogram that collections originating from various parts of the study area did not form well-defined distinct clusters. They were interspersed with each other, indicating no association between RAPD pattern and the area of collection of accessions. This however, contrasted with the finding of Ge et al. (2013) who used SSR markers to obtain clusters among Chinese eggplant accessions that resulted in clades corresponding to the geographic divisions.

The present data revealed that *S. dasyphyllum* is more closely related to *S. macrocarpon* than to *S. melongena* as evident in cluster I. This observation is in agreement with the findings of Mace et al. (1999), and Isshiki et al. (2008). These workers used AFLP markers to determine the taxonomic position of *S. dasyphyllum* and *S. macrocarpon* both of series Macrocarpa outside section Melongena which comprises *S. melongena*. According to Mace et al. (1999), this close relationship between *S. macrocarpon* and *S. dasyphyllum* is also supported by earlier findings of Jaeger (1986) who considered *S. macrocarpon* to be a domesticated modification of the wild plants known as *S. dasyphyllum*. Mace et al. (1999) stated further that Jaeger (1986) then assigned the wild form of a subspecies status under *S. macrocarpon*, the earlier name.

The occurrence of most samples of *S. gilo* and two samples (out of three) of *S. melongena* in cluster IV is an indication of close relatedness and possibility of having a common ancestor. Occurrence of *S. incanum* together with *S. melongena* still in cluster IV also indicates relatedness and probably progenitors from a common ancestral lineage. This observation of closeness between *S. incanum* and *S. melongena* supports the earlier finding of Sakata and Lester (1994) that used chloroplast DNA, Karihaloo et al. (1995), Furini and Wunder (2004) and Singh et al. (2006). In fact, Karihaloo et al. (1995) had earlier observed that wild forms of *S. incanum* are regarded as belonging to the same species as *S. melongena*. Singh et al. (2006) also stated that at the species level the cultivable type of *S. melongena* is more closely related to *S. incanum* followed by *S. viarum* whereas *S. surattence* and *S. nigrum* showed a closer association among themselves in comparison with the cultivated *S. melongena. S. scabrum* and *S. nigrum* occur together in both Clusters III and V, an indication of similarity between the two. The implication of this is that they are only distantly related to *S. melongena* and are more closely related to each other.

The level of polymorphism observed in the present study was high going by the coefficient of variation. The correlation coefficient 0.95 for the highest similarity between genotypes and the least 0.50 exhibited a good separation from a conserved region of the genome. This is an indication that eggplant *Solanum* has a wide and diverse genetic base.

These results agreed with those obtained by previous workers on *Solanum* e.g. Furini and Wunder (2004), Singh et al. (2006) and Levin et al. (2006). However, these are not in agreement with some earlier workers; for instance, Karihaloo and Gottlieb, (1995) studied variation among the cultivated and weedy taxa of *S. melongena* by allozymes and RAPD analyses; also Ge et al. (2013) examined the genetic diversity and relationships among eggplant accessions collected from seven areas in China using SSR markers. These authors observed little or moderate amount of genetic polymer-phism among the genotypes studied; even Karihaloo and Gottlieb (1995) suggested the existence of a very small gene pool from

which the cultivated forms of *S. melongena* arose.

However, RAPD has some disadvantages which may affect the reliability of these results. For example, it is non-reproducible; they are dominant thereby making it impossible to distinguish between homozygosity and heterozygosity, and also RAPD results can be difficult to interpret. To overcome these, Ali et al. (2013) for example, analyzed the diversity of Chinese eggplant using inter-simple sequence repeat (ISSR) and RAPD procedures. The results showed that ISSR markers were more effective than RAPD markers for detecting genetic diversity.

Notwithstanding, the overall results of the present study were satisfactory enough in terms of their statistical values and concordance with previously published data. However, the accuracy of the clustering result may be increased by increasing the data and sample numbers of eggplant accessions as well as employing other better markers such as SSR, AFLP, ISSR, etc, in the analysis.

Conclusion

The study provides species database of the vegetable, *Solanum* and related species in Southwestern Nigeria and by extension in the country as a whole with emphasis on variation patterns which is a major contribution to global biodiversity information system. From the study also, it is evident that RAPD and other discontinuous markers can be made use of as a means of genetic distances to establish *Solanum* taxonomy as well as phylogenetic relationships among taxa. Detection of genetic differences and discrimination of genetic relationship between *Solanum* species are for sustainable utilization and conservation of plant genetic resources.

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Variability assessment of seed traits in *Jatropha curcas* L. for improvement of oil yield

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***Jatropha curcas* has received considerable attention from researchers as a potential source of non-edible vegetable oil which is eminently suitable for production of liquid bio fuel, meeting international standards. For any tree improvement and breeding programme, study of variation among the populations is prerequisite as it helps in the detection of relative performance of various traits of economic value. 28 candidate plus trees were selected from the states of Jharkhand, West Bengal and Bihar in Eastern India. Nine seed parameters were measured and their genetic values and correlation was calculated to serve as base information for further improvement and breeding of *J. curcas* in Eastern India. Significant genetic differences exist in all the seed characteristics among the different candidate plus trees of *J. curcas*. Positive direct effect of seed width and protein content was observed on seed oil content. Indirect effect of seed traits via seed width was positive. Thus, seed width can be taken as a criterion for selecting trees with good oil yield. The variations in most studied parameters were under genotypic control among seed sources. Highest coefficient of variation was found for seed oil content emphasizing the need of wide scale screening and selection of superior genotypes to capture the existing variability.**

Key words: Candidate plus trees, correlation, genetic gain, heritability, seed oil.

INTRODUCTION

Jatropha curcas L. is a small multipurpose tree with valuable attributes and considerable economic potential that grows in the equatorial Americas and has spread over other tropical countries cultivated worldwide for production of non-edible seed oil. In recent years *J. curcas* has drawn attention as a source of seed oil that can provide an economically viable substitute for motor fuel (Openshaw, 2000; Adebowale and Adedire, 2006; Chen et al., 2006). *J. curcas* is among the most suitable tree species for production of biodiesel as it can be cultivated as a quick yielding plant even in problem soils and adverse environmental conditions. Potentially high yield of oil per unit land area in *J. curcas* is second only to oil palm (Fairless, 2007). Furthermore, the quality of oil in its seeds is suitable for production of biodiesel as they contain more than 75% unsaturated fatty acids (Biello, 2009). Among the oil bearing trees, *J. curcas* is desired due to its drought hardiness, rapid growth, easy propagation, small gestation period, wide adaptability, production on good and degraded soils and optimum plant size that makes seed collection more convenient (Jones and Miller, 1991; Francis et al., 2005).

Worldwide introduction of *J. curcas* for varied purposes had met with limited success due to unreliable and low seed set as well as oil yields resulting in poor economic returns (Singh et al., 2010). *J. curcas* is a wild species and no varieties with desirable traits for specific growing conditions are available, which makes its cultivation a risky business (Jongschaap et al., 2007). The crop is also

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characterized by variable and unpredictable yield due to unidentified reasons (Ginwal et al., 2004). The positive attributes of this plant are not fully understood in terms of breeding and utilization (Fairless, 2007) which limits its large-scale cultivation and warrants the need for genetic improvement and breeding of superior genotypes of the species.

Selection is the most important activity in all tree breeding programmes (Zobel and Talbert, 1984). *J. curcas* being highly cross-pollinated species is anticipated to contain wide genetic variability offering significant scope for selecting superior genotypes which will help in the improvement of productivity. Knowledge of genetic relationship and variation in the species is a prerequisite in any breeding programme because it permits the organization of germplasm, including elite lines and provides for more efficient selection (Karp and Edwards, 1998). However, the major constraint in achieving higher quality oil yield of *J. curcas* is lack of information about its genetic variability, oil composition and absence of suitable ideotypes for different cropping systems.

Plants, in general, might be expected to maintain high levels of genetic variation within their populations since their sessile nature often leads to the evolution of locally adapted ecotypes (Antonovics, 1971; Bradshaw, 1972; Jain and Bradshaw, 1966). This variation may be utilized in selection and improvement of the species. Gaining insights into the genetic variability of *J. curcas* provenances and elucidation of correlations between the genetic parameters and biochemical characteristics of seed provenances collected from different regions of the world would be a critical input for the selection of appropriate genotypes for cultivation and breeding purposes (Basha et al., 2009). Modern techniques have accelerated characterization of *J. curcas* germplasm at molecular level (Singh et al., 2010; Xu et al., 2012) and even whole genome sequencing (Sato et al., 2010). However, information regarding the extent and pattern of genetic variation in *J. curcas* population is limited barring a few recent studies (Kaushik et al., 2007; Rao et al., 2008; Gairola et al., 2011; Tripathi et al., 2013; Brasileiro et al., 2013). Therefore, genetic and correlation studies among the seed traits were carried out in candidate plus trees of *J. curcas* selected from a wide geographical range in three Eastern Indian states aiming at utilization in oil yield improvement.

MATERIALS AND METHODS

Twenty eight (28) Candidate Plus Trees (CPTs) of *J. curcas* were selected during 2007-2010 from naturalized areas of Eastern India (states of Bihar, West Bengal and Jharkhand) on the basis of seed bearing quality, crown size and tree health. The details of the selected plus trees are given in Table 1.Seeds of plus trees were collected to measure seed traits under laboratory conditions. These included seed length (cm), seed width (cm), hundred seed weight (g), 2D surface area (cm^2), aspect ratio, germination percentage (%), total soluble carbohydrate (mg/g), total soluble protein (mg/g) and seed oil percentage (%).

Total soluble carbohydrate and total soluble protein were as estimated according to standard methods of Dubois et al. (1956) and Lowry et al. (1951) on UV-Vis spectrophotometer (Pharos, Merck Germany). Seed oil content from seed kernel sample was studied using Soxhlet apparatus. 100 g of seed were oven dried at 40°C overnight before the seeds were broken to get the kernels. 10 g of these kernels were finely chopped, wrapped in Whatman's Filter paper Number 2 and kept in the middle chamber Sox let's apparatus for oil extraction. Empty weight of the boiling flask was taken. In the boiling flask, petroleum ether (boiling range 60 to 80°C) was maintained at about 70°C. The ether vapors after getting condensed in the condenser chamber got collected as liquid ether in the middle chamber. The kernels came in contact with ether and the oil in the kernels got dissolved with ether which led to the rise in ether level in the middle chamber. Oil mixed ether was then poured down into the lower boiling flask and the entire process was repeated once again which allowed ether to get evaporated and not the oil as the later had higher boiling range. The lower boiling flask was kept in boiling water bath for about 35 min to expel the ether and separate the oil. Final weight of the boiling flask with oil was taken and net weight of *Jatropha* oil obtained was calculated from the difference of the two weights of the boiling flask. Percentage of oil in seeds was measured as oil content percentage = weight of extracted oil (g)/sample weight x 100.

The data of seed traits was subjected to statistical analysis firstly to test the significance among the various CPTs by analysis of variance. The Statistical Package for Agriculture Research, version 2.0 (SPAR 2.0) was used for calculating various variability parameters (phenotypic coefficient of variability, genotypic coefficient of variability, heritability, expected genetic advance and genetic gain), correlation among different traits, path and Euclidean clusters analysis.

RESULTS AND DISCUSSION

Seed contain a lot of variation from one origin to another origin with regards to morphological variation and physiological differences which could be genetic in nature as a result of adaptation to diverse environmental condition prevailing throughout their distributional range (Mathur et al., 1984). Apart from age, vigour, crown exposure and genotype of mother tree, soil and climate of the place of seed origin are important factors affecting the seed traits (Salazar and Quesada, 1987). Due to its wide geographical distribution, there is considerable scope of genetic variation in *J. curcas* seeds, which are the principal means of propagation. Significant trait differences were observed in seed characters namely: seed morphology and oil content as well as in growth characters including seed yield in the progeny trial of *J*. curcas for evaluating genetic association, and variability in seed and growth characters (Rao et al., 2008). Variability in seed traits and oil content of 24 accessions of *J*. *curcas* collected from different agroclimatic zones of Haryana state, India were assessed to record significant differences in seed size, 100-seed weight and oil content between accessions (Kaushik et al., 2007).

We also observed significant variation among the candidate plus trees for all the seed parameters (Table 2).

Table 1. Location of selected candidate plus trees of *Jatropha curcas* in Eastern India.

CPT	Locality (District)	Latitude	Longitude	State
J_1	Katangdri (Ranchi)	23° 34' 38.76"	85° 18' 34"	Jharkhand
J_2	Hutar (Khunti)	23° 7'54.2"	85° 16' 11"	Jharkhand
J_3	Banai (Gumla)	22°52' 15.88"	84° 49' 27.14"	Jharkhand
J_4	Banai Bindratoli(Gumla)	22°52' 15.88"	84°49' 30"	Jharkhand
J_5	Patura Dhauntatoli(Gumla)	22° 52' 12.43"	84° 49' 30"	Jharkhand
J_6	Purnatoli (Simdega)	22° 41' 53.53"	84° 41' 56.24"	Jharkhand
J_7	Fikpani(Simdega)	22° 41' 55.77"	84° 42'1.15"	Jharkhand
J_8	Jamtoli(Simdega)	22° 43' 40.9"	84° 41' 55.2"	Jharkhand
J_9	Pandripani Karanjtoli(Simdega)	22° 30' 0.7"	84° 30' 40.33"	Jharkhand
J_{10}	Silaphari Hundratoli (Gumla)	22° 2' 50.63"	84° 32' 31.45"	Jharkhand
J_{11}	Hurudag (Hazaribagh)	24° 6' 32.53"	85°12' 13.14"	Jharkhand
J_{12}	Barhi (Hazaribagh)	24° 18' 16.37"	85°25' 18.79"	Jharkhand
J_{13}	Motileda Bermasia (Giridih)	24° 18' 10.68"	86° 21' 46.02"	Jharkhand
J_{14}	Palunjia (Giridih)	24° 16' 10.31"	85° 55' 54.23"	Jharkhand
J_{15}	Bardih (Bokaro)	23° 40' 7.15"	86° 3' 19.83"	Jharkhand
J_{16}	Dantu (Bokaro)	23°36' 16.94"	85°56' 12.63"	Jharkhand
J_{17}	Baghmundi (Purulia)	23° 11' 52.86"	86° 2' 38.82"	West Bengal
J_{18}	Awasgarh (Midnapore)	22°25' 15.13"	87° 19' 33.91"	West Bengal
J_{19}	Salboni (Midnapore)	22°38' 32.12"	87° 19' 8.42"	West Bengal
J_{20}	Pathrajuri (Midnapore)	22° 32' 27.08"	87° 18' 32.64"	West Bengal
J_{21}	Bhadua (Midnapore)	22° 17' 25.28"	86° 56' 59.61"	West Bengal
J_{22}	Satkui (Midnapore)	22° 22' 34.12"	87°20' 33.36"	West Bengal
J_{23}	Binpur (Midnapore)	22° 34' 38.17"	87° 0' 14.96"	West Bengal
J_{24}	Rajgir Road (Nalanda)	25° 1' 54.43"	85° 25' 10.49"	Bihar
J_{25}	Rajgir (Nalanda)	25° 1' 46.0"	85° 24' 59.83"	Bihar
J_{26}	Kenar (Gaya)	24° 46' 2.87"	85° 16' 10.77"	Bihar
J_{27}	Berhibigha (Gaya)	24° 48' 7.83"	85° 14' 37.62"	Bihar
J_{28}	Wajirganj (Gaya)	24° 47' 53.14"	85° 2' 4.12	Bihar

J_{17} depicted the maximum seed length, seed width, 2D surface area, aspect ratio and germination percentage. Maximum 100-seed weight was found for J_{19} (74.35) which were statistically at par with J_{17}, J_{23}, J_{16}, J_3, J_{15}, J_{24}, J_9 and J_1. J_{16} (17.13) followed by J_{18} exhibited highest value for total soluble carbohydrate. Maximum seed oil percentage was estimated in J_{27} (45.38) and the minimum value in J_{22} (27.77) (Table 2). Wide variation for seed traits are in conformity with earlier report in *J. curcas* (Anonymous, 2005; Ginwal et al., 2004; Kumar et al., 2003) and other tree species for example Japanese black pine (Miyata et al., 1991), and *Dalbergia sissoo* (Singh and Pokhriyal, 2001). Variations observed in seed traits are expected to be genetic in nature as a result of adaption to diverse environmental conditions where CPT selection was made.

The proportion of total variation, which is heritable, is termed as heritability in broad sense (Lush, 1937). Knowledge of its magnitude gives an idea about scope of effecting genetic improvement through selection. Heritability in broad sense may give useful indication about the relative value of selection in the material at hand, to arrive at a more reliable conclusion. Variability and genetic parameters of seed and germination traits have been presented in Table 3. Highest coefficient of variation was exhibited for seed oil percentage (11.58) and the lowest for protein content (0.12). Total soluble carbohydrate depicted the maximum phenotypic (63.93) and genotypic coefficient of variability (63.92), heritability (0.999) and genetic gain (131.66). Seed width exhibited the lowest phenotypic (4.34) and genotypic coefficient of variability (4.20), genetic advance (0.09) and genetic gain (8.04). Lowest heritability was found for seed oil percentage (0.302).

Heritability and associated genetic gain should be considered jointly. Heritability estimates along with genetic gain is more useful than the heritability alone in predicting the resultant effect for selecting the best genotype for given trait (Johanson et al., 1955; Volker et al., 1990). Therefore, a heritability estimate alone does

Table 2. Variation in seed traits of candidate plus trees of *J. curcas.* Data in parentheses are transformed values.

CPT	Seed length (cm)	Seed width (cm)	100-Seed weight (g)	2D Surface area (cm^2)	Aspect ratio	Germination percentage (%)	Total carbohydrate (mg/g)	Total soluble protein (mg/g)	Seed oil content (%)
J_1	1.52	1.06	46.48	10.11	1.43	54 (46.30)	2.52	14.8	42.17(37.40)
J_2	1.61	1.07	53.50	10.89	1.50	64(52.14)	10.02	19.28	40.80(36.60)
J_3	1.83	1.17	69.20	13.45	1.56	78(61.05)	1.88	11.63	40.60(36.48)
J_4	1.45	1.06	40.10	9.61	1.37	51(44.58)	2.86	13.67	44.30(36.63)
J_5	1.69	1.10	59.28	11.66	1.53	70(55.80)	2.54	14.83	38.08(35.00)
J_6	1.77	1.13	57.05	12.53	1.56	70(55.80)	3.88	13.70	38.32(35.15)
J_7	1.78	1.09	59.70	12.15	1.64	68(54.56)	7.35	15.81	37.35(34.56)
J_8	1.58	1.11	65.00	11.03	1.43	74(58.38)	8.88	17.15	40.88(36.64)
J_9	1.77	1.18	67.65	13.14	1.50	72(57.06)	1.21	11.32	40.36(36.34)
J_{10}	1.57	1.04	44.23	10.22	1.50	52(45.15)	6.33	17.11	39.88(36.06)
J_{11}	1.68	1.11	63.40	11.69	1.51	72(57.06)	2.15	12.06	41.25(36.86)
J_{12}	1.78	1.13	51.80	12.66	1.57	62(50.45)	8.72	18.03	43.92(38.41)
J_{13}	1.77	1.11	66.63	12.32	1.59	75(59.01)	3.76	15.59	35.01(33.18)
J_{14}	1.67	1.08	58.38	11.26	1.55	69(55.18)	8.94	19.01	44.11(38.52)
J_{15}	1.79	1.14	68.98	12.78	1.58	70(55.78)	10.4	19.35	42.59(37.64)
J_{16}	1.70	1.21	70.10	12.92	1.40	82(63.90)	17.13	19.24	35.74(33.61)
J_{17}	1.85	1.22	73.50	14.18	1.51	84(65.44)	4.02	13.72	42.59(37.64)
J_{18}	1.73	1.11	60.33	12.08	1.56	68(54.56)	16.27	18.09	35.74(32.27)
J_{19}	1.77	1.22	74.35	13.55	1.44	82(63.93)	6.09	13.93	42.31(37.48)
J_{20}	1.79	1.13	62.28	12.65	1.59	71(56.42)	5.96	13.01	43.26(38.03)
J_{21}	1.80	1.12	62.15	12.60	1.61	72(57.06)	2.25	11.06	37.10(34.42)
J_{22}	1.44	1.08	49.58	9.80	1.34	58(48.61)	13.04	16.45	27.77(28.70)
J_{23}	1.79	1.14	71.15	12.78	1.58	79(61.74)	9.32	19.16	38.97(35.53)
J_{24}	1.78	1.14	68.28	12.72	1.56	69(55.18)	5.95	14.48	39.34(35.74)
J_{25}	1.61	1.07	60.63	10.73	1.51	58(48.61)	6.93	17.21	38.76(35.40)
J_{26}	1.77	1.12	63.98	12.41	1.58	55(46.87)	6.22	17.89	36.11(33.83)
J_{27}	1.80	1.11	63.75	12.56	1.62	54(46.30)	7.78	18.66	45.38(39.25)
J_{28}	1.49	1.13	65.65	10.55	1.32	56(47.45)	1.99	10.24	45.12(39.10)
$LSD_{0.05}$	0.018	0.0172	8.14	0.211	0.031	2.242	0.106	0.027	6.538

not necessarily mean an increased genetic advance. High heritability (broad sense) may be due to non-additive gene action so it shall be reliable only if accompanied by high genetic gain (Rawat and Nautiyal, 2007). In the present study, higher heritability values of the seed traits were generally accompanied by high genetic gain as earlier reported for seed weight in *Graewia optiva* (Uniyal, 1998) and *Celtis australis* (Jain, 1982). This indicates that high heritability leads to increased genetic gain if sufficient genetic variability exists in the germplasm.

Seed oil content variation is more widely reported not only in annual crops but also in a wide variety of trees borne oil seed (Johansson et al., 1997; Kaura et al., 1998; O'Neill et al., 2003; Vollmann et al., 2007). The variation found in oil content in the present study along with other seed morphological attributes presents us with a viable selection alternative at a very early stage from base seed material. Oil content is greatly influenced by environmental factors such as soil conditions (Srivastava, 1999). Phosphorus has been found to be the main requirement for increase in oil yield in the case of castor beans growing in East Africa (Geus and Jan, 1973). A drier climate is supposed to improve the oil yield in *J. curcas* seeds (Jones and Miller, 1991). These findings support the present research that oil content is mainly affected by environment conditions as compared to genetic makeup. We also recorded highest coefficient of variation for seed oil content, however it possessed lowest heritability. Heritability was high for total soluble carbohydrates, protein content, 100-seed weight, seed length and seed width, which shows that through clonal

Table 3. Mean coefficient of variation (CV (%) and genetic estimates of seed traits of *J. curcas*

Character	Range	Mean	CV (%)	Coefficient of variability		Heritability	Genetic advance	Genetic gain (%)
				Phenotypic	Genotypic			
Seed Length	1.43 - 1.86	1.698	0.751	7.052	7.012	0.989	0.24	14.134
Seed Width	1.00 - 1.23	1.12	1.084	4.338	4.2	0.937	0.09	8.036
100-seed weight	40.0 - 74.6	61.323	9.389	16.425	13.478	0.673	13.97	22.603
2D Surface Area	9.56 - 14.37	11.964	1.246	10.118	10.041	0.985	2.46	20.56
Aspect Ratio	1.31 - 1.65	1.517	1.442	5.783	5.601	0.938	0.17	11.206
Germination (%)	50.0 - 85.0	67.464	2.349	14.46	14.269	0.974	19.57	29.008
Total soluble carbohydrate	1.18 - 17.5	6.585	1.142	63.932	63.922	0.999	8.67	131.663
Total S. Protein	10.22 - 19.38	15.589	0.122	18.258	18.258	0.999	5.86	37.591
Seed Oil Content	27.64 - 45.42	39.922	11.581	13.863	7.62	0.302	3.44	8.617

Table 4. Simple Correlation between seed traits and germination percentage in *J. curcas.*

Character	Correlation with germination percentage
Seed Length	0.6080*
Seed Width	0.7277*
100-seed weight	0.6468*
2D Surface Area	0.7306*
Aspect Ratio	0.211
Total soluble carbohydrate	0.0786
Total soluble protein	-0.1187
Seed Oil Content	-0.0493

*Significant at 1%.

propagation, we can trap these qualities of mother plant. Manga and Sen (1996) in *Prosopis cineraria* and Mahadevan et al. (1999) in *Casuarina equisetifolia* have also recorded similar heritability of seed traits. Correlation is one of the important biometrical tools, which measure the degree and magnitude of association among different traits. In tree improvement programme, a clear understanding of association among different traits is of great importance as it illustrates weather the choice of one character confirm the appearance or disappearance of other. Significantly and positively correlation of 2D surface area (0.7306), seed width (0.7277), 100-seed weight (0.6468) and seed length (0.6080) were recorded with germination percentage at 1% level of significance. A negative but non-significant correlation was found of seed protein content and seed oil percentage with germination percentage (Table 4). Seed length, seed width and 100- seed weight has significant simple, phenotypic and genotypic correlation with germination percentage in the present investigation as previously reported in Douglas fir (Clair and Adams, 1991) and *P. cineraria* (Manga and Sen, 1995).

Proper utilization of observed variation in a species depends upon the knowledge the extent of variation and its cause, whether it is due to genetic (heritable) or the environmental and phenotypic (non heritable) factors. The related magnitude of these components determines the genetic properties of any particular species (Jain, 1982). Release of high yielding clones/cultivars cannot be done without ascertaining the magnitude of variation present in the available germplasm, interdependence of growth pattern with yield, extent of environmental influence on these factors, heritability and genetic gain of the material. Considerable genetic variation in growth, chemical composition of seed and seed traits at the level of provenance, variety or progeny has been reported in most outcrossing multipurpose tree species such as *Albizia*, *Acacia*, and *Prosopis* (Costa et al., 2005; Wanyancha et al., 1994; El Amin et al., 2006; Goel and Behl, 2001). We also recorded highly significant and positive phenotypic as well as genotypic correlation of seed length, seed width, 100-seed weight and 2D surface area with germination percentage (Table 5). 2D surface area (0.732) had the maximum phenotypic correlation followed by seed width (0.730), 100-seed weight (0.653) and seed length (0.608). 100-seed weight (0.803) depicted the maximum genotypic correlation followed by seed width (0.761), 2D surface area (0.745) and seed length (0.619). Protein content and seed oil content showed non-significant and negative phenotypic and genotypic correlation. No combination had significant environmental correlation. Relative proportion of phenotypic, genotypic and environmental correlations for seed morphological and biochemical characteristics presents a contrasting scenario (Figure 1a and b) where in the case of the former genotypic correlations were stronger and significant while environmental correlations were strong though non-significant for seed oil and total protein. Thus, genotypic-/phenotypic variations scored over environmental variation. When more variables are included in the correlation studies, the inherent association becomes more complex. Under such circumstances, to understand the specific

Table 5. Phenotypic, genotypic and environmental correlation between seed traits and germination percentage in *J. curcas.*

Character	Correlation with germination percentage		
	Phenotypic	Genotypic	Environmental
Seed Length	0.608*	0.619*	0.057
Seed Width	0.730*	0.761*	0.074
100-seed weight	0.653*	0.803*	0.022
2D Surface area	0.732*	0.745*	0.104
Aspect ratio	0.211	0.222	-0.044
Total soluble carbohydrate	0.079	0.079	0.060
Total soluble protein	-0.119	-0.120	0.082
Seed oil content	-0.049	-0.131	0.164

forces in building up of total correlation and to resort direct and indirect effects of different contributing traits on important desirable traits, path coefficient analysis is imperative. Perusal of Table 6 reveals that aspect ratio (4.9638) resulted in maximum direct effect on seed oil content, while seed length (-5.9372) depicted the maximum negative indirect effect. Seed length depicted the negative indirect effect on seed oil content by 100-seed weight, 2D surface area and germination percentage and positive indirect effect by seed width, aspect ratio, total soluble carbohydrate and protein content. Seed width showed positive direct effect (4.4862) on seed oil content and positive indirect effect via aspect ratio; however negative indirect effect via other seed traits. 100-seed weight depicted negative direct effect (-0.1241) on seed oil content. It revealed positive indirect effect via seed width and aspect ratio and via others negative indirect effects. 2D surface area revealed the negative direct effect (-0.4944) on seed oil content. It had positive indirect effect via seed width, aspect ratio and total soluble carbohydrate and negative indirect effect via other remaining traits. Aspect ratio resulted in positive indirect effects by seed width, aspect ratio, protein content and total soluble carbohydrate and negative indirect effect via other remaining traits. Germination percentage depicted the negative direct effect (-0.3556) on seed oil content and positive indirect effect via seed width and aspect ratio and negative indirect effect via other remaining traits. Total soluble carbohydrate resulted in negative direct effect (-1.0007) on seed oil content. It showed positive direct effect via seed width, 2D surface area and protein content. Via other traits, there were negative indirect effects. Protein content revealed positive direct effect (0.6484) on seed oil content. It had positive indirect effects via 100-seed weight, 2D surface area, aspect ratio and germination percentage; however negative indirect effects via seed length, seed width and total soluble carbohydrate.

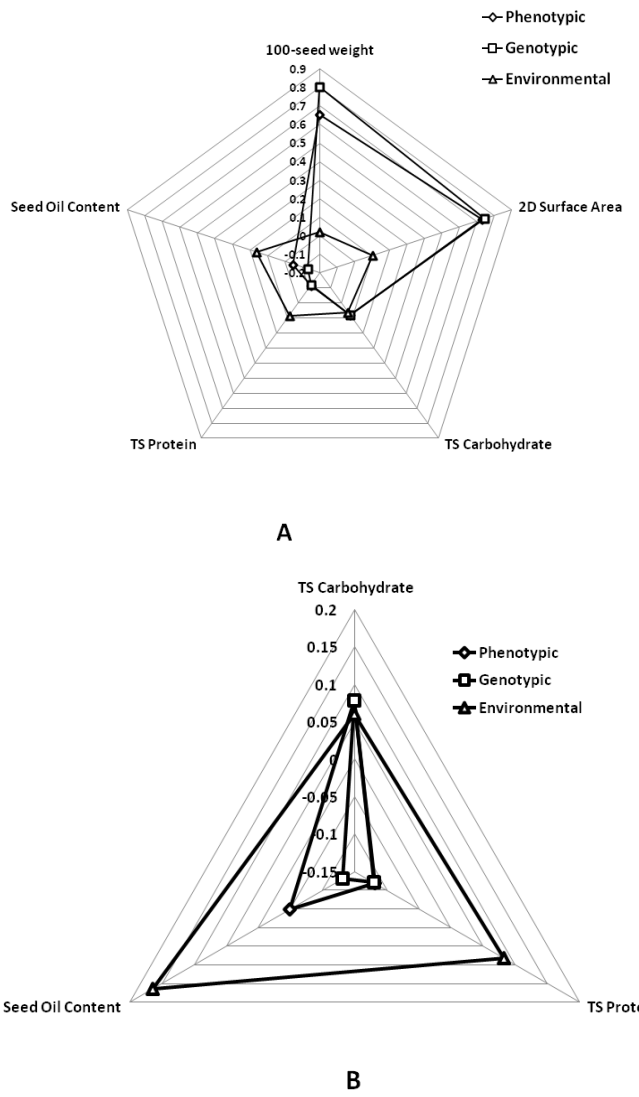

Figure 1. A. Relative proportion of phenotypic, genotypic and environmental correlations for seed morphological characteristics. **B.** Relative proportion of phenotypic, genotypic and environmental correlations for seed oil and other biochemical traits.

Reliable classification of accessions along with identify-cation of core subsets among the accessions with future utility for specific breeding purposes can be expedited through analysis of genetic diversity in germplasm collec-tions. 28 CPTs of *J. curcas* were grouped in four clusters using non-hierarchical Euclidean Cluster Analysis (Table 7). However, *K*-means clusters clustering pattern in the present study revealed trees from different geo-graphic regions grouped together in a cluster and trees from the same geographical areas placed in different clusters indicating separation of geographical diversity from inherent genetic diversity of the germplasm. *K*-means clustering is done to understand the trend of evolution and

Table 6. Path coefficient analysis results showing direct (in italics) and indirect effects of seed traits on seed oil content in *J. curcas.*

Character	Seed length	Seed width	100-seed weight	2D Surface area	Aspect ratio	Germination (%)	TS Carbohydrate	TS Protein	Total effect on seed oil content
Seed length	*-5.9372*	2.7287	-0.0882	-0.4651	4.0357	-0.2201	0.0390	0.0001	0.0929
Seed width	-3.6112	*4.4862*	-0.1054	-0.4156	0.1637	-0.2704	-0.0151	-0.154	0.0787
100-seed weight	-4.2189	3.8082	*-0.1241*	-0.4150	1.3803	-0.2857	-0.0216	-0.074	0.0496
2D surface area	-5.5856	3.7715	-0.1042	*-0.4944*	2.8184	-0.2649	0.0257	-00665	0.1001
Aspect ratio	-04.8271	0.1479	-0.0345	-0.2807	*4.9638*	-0.0798	0.0500	0.1187	0.0583
Germination (%)	-3.6758	3.4121	-0.0997	-0.3683	1.1135	*-0.3556*	-0.0795	-0.078	-0.1313
TS carbohydrate	0.2314	0.0676	-0.0027	0.0127	-0.2479	-0.0283	*-1.0007*	0.5071	-0.1810
TS protein	-0.0012	-1.0617	0.0141	0.0507	0.9086	0.0428	-0.7826	*0.6484*	-0.1810

Table 7. Composition of Euclidean clusters and cluster mean values obtained by *K*-means Non-hierarchical clustering for seed and oil traits in *J. curcas.*

Cluster	Number of CPTs	CPT Codes	Seed oil (%)
I	10	J_4, J_6, J_8, J_{12}, J_{20}, J_{21}, J_{22}, J_{25}, J_{27}	40.01
II	6	J_1, J_2, J_3, J_5, J_7, J_{14}	37.58
III	8	J_9, J_{11}, J_{16}, J_{18}, J_{19}, J_{23}, J_{24}, J_{28}	41.34
IV	4	J_{10}, J_{13}, J_{15}, J_{26}	40.36

choose genetically diverse parents for obtaining desirable recombination (Tams et al. 2006). Intra-cluster distances were lower (maximum 1.59 in III and minimum 0.853 in I) than that of the inter-cluster distances indicating the genetic similarly among the members within clusters. Maximum inter-cluster distance (6.323) between cluster I and IV suggests extensive genetic diversity between the trees in these groups and selecting of parents from these clusters would prove useful in developing novel hybrids. Selection of trees as parents from clusters with low inter-cluster distances for example II and III (2.162) should be avoided.

Present study suggests positive direct effect of seed width and protein content on seed oil content. Positive indirect effect of seed traits via seed width suggests that seed width can be taken as a criterion for selecting trees with good oil yield. It is clear that considerable genetic differences exist in all the seed characteristics among the different CPTs of *J. curcas*.

J_{17} was found as a superior seed source on the basis of seed morphological characters. However, for current trait of interest in *J. curcas* that is seed oil, J_{27} exhibited superiority. The variations in most studied parameters are under genotypic control among seed sources. Highest coefficient of variation was found for seed oil content emphasizing the need of wide scale screening and selection of superior genotypes to capture the existing variability. However, low heritability of seed oil makes the task difficult for breeders.

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