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RESEARCH ARTICLE

SEED SOURCE VARIABILITY IN HALF SIB FAMILIES OF *DIOSPYROS MELANOXYLON* ROXB. UNDER OPEN FIELD CONDITION

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Abstract

The performance of thirty phenotypically superior trees in the natural distribution zones of Madhya Pradesh, Chhattisgarh and Jharkhand were studies for seed source variation in *Diospyros melanoxylon* at School of Forestry and Environment, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad. *Diospyros melanoxylon* Roxb. is commonly known as tendu, belonging to the family Ebenaceae. Based on the relative magnitude of D^2 values thirty superior trees progenies were grouped into six clusters on the basis of morphological and biomass traits. Cluster I exhibited the highest number of eight superior tree progenies. The clustering pattern in this study revealed that superior tree progenies from different geographic region were grouped together in a cluster and vice-versa suggesting that geographical diversity did not necessarily represent the genetic diversity. The contribution of different traits towards diversity revealed that seedling height contributed the maximum towards divergence. The study also showed that the phenotypic coefficient of variation (PCV) was higher than the corresponding genotypic coefficient of variation (GCV) for all the morphological and biomass traits which revealed that the traits were sensitive to environmental fluctuations. The traits with low genetic gain and high heritability indicate that the expression is possibly controlled by intra and inter allelic interactions. The hybridization between the more diverse genotypes of *Diospyros melanoxylon* can produce genotypes with high heterotic vigor.

INTRODUCTION

The last few decades have witnessed the emergence of wide spread concern to solve the major problem of the erosion of genetic resources. The only realistic solution of the continuing loss of plant germplasm is the genetic conservation; that is the collection and systematic preservation of germplasm in gene resource centers with a wide representation of genetic resources of the species as practicable. In order to establish priorities for the conservation and improvement of tree genetic resources understanding of the diversity among and between tree populations is required. Determining the amount, cause and nature of variation present in the species of interest is the first step towards any improvement work. All differences among trees are the result of three things *viz.*, different environment in which tree is growing, the genetic differences among trees and interaction between tree genotype and environment in which they grow. Continuous development is possible if variation exists in a species. Variations are essential for adaptation and improvement and the amount of variation determine the potential for improving species through breeding programmes. In natural population of a species, the presence of land masses, water bodies and mountains cause variation. High genetic variation within and among populations has been demonstrated and this distribution of variation and evolutionary histories can lead to the recommendations of future breeding programmes **(Namkoong, 1984).**

Diospyros melanoxylon Roxb. is commonly known as tendu belonging to the family Ebenaceae. *Diospyros melanoxylon* is mainly found in north tropical moist deciduous forests, southern tropical dry deciduous forests and northern tropical dry deciduous forests. Tendu tree have very important role in the socio-economic uplifment of tribal populations

Tendu leaf constitutes the body of the bidi, the mixture of small pieces of blended tobacco wrapped spirally winded around the tobacco. The leaf of the tree contains valuable flavones. The pentacyclic triterpines found in the leaves possess antimicrobial properties **(Venkata *et.al.*, 2004),** while the bark shows anti hyperglycemic activity. The bark of the *Diospyros* species found in India has been determined to have significant anti-plasmodial effects against *Plasmodium falciparum* which causes malaria in humans **(Satyanarayana and Wright 2008)**. Fruits are useful in urinary complaints and is a germicide. Dried flowers are reported to be useful in urinary, skin and blood diseases **(Hocking, 1993)**. The seeds are cure for mental disorders, palpitation of heart and nervous breakdown. Bark of this tree is used in diarrhea. The bark is burnt by tribals to “cure” small-pox. Besides being the source of Indian ebony, its wood is also utilized for making boxes, combs, ploughs and beams **(Rathore, 1970)**. The heart wood is black and is used for making furniture. The occurrence of this species over a wide geographic range encompassing a great diversity of edaphoclimatic conditions of its habitat is expected to be reflected in the genetic constitution of its diverse population **(Searle, 1961)**.The species therefore offers an opportunity, for studying variation and also to select the superior seed sources for adaptability and growth. Due to longer rotation period of the tree, there is very less information available on its genetic improvement Therefore, an effort was made to study the genetic differentiation in superior tree progenies to identify the diverse genotype to use further in hybridization programme.

MATERIALS AND METHOD

The present study was conducted at School of Forestry and Environment, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad to estimate the genetic divergence in seedling morphology and biomass characters of *Diospyros melanoxylon* collected from different locations of Madhya Pradesh, Chhattisgarh and Jharkhand. The area is situated at 28° 87' N latitude and 81° 15' E longitude at an elevation of 98 meters above mean sea level on Northern aspect. On an average the area receives an annual rainfall of 1100 cm, most of which happens during monsoon season. Mature fruits from different parts of the crown for the present study was procured by selecting, the thirty phenotypically superior trees in the natural distribution zones of Madhya Pradesh, Chhattisgarh and Jharkhand on the basis of different morphological, fruit and seed character mentioned in Table 1 viz. a) Tree height, b) Tree diameter, c) Number of seeds per fruit, d) seed length and e) Seed diameter. Five phenotypically superior trees of same size and free from any disease and pest were selected and observations were made. Out of these, the best one was considered as plus tree. Uniform, healthy seeds from well mature pods of plus trees from thirty representing stands were collected to constitute the seed lot for each family. The details of the plus tree are given in Table 1. For experimental purpose, the seeds were sown in a randomized block design replicating thrice in poly-bags of size 10 × 25 cm filled with a mixture of soil, sand and Farm Yard Manure (1:1:1) at a depth of 0.5 cm. The observations were made for twelve months old seedlings for different morphological and biomass traits viz., seedling height, seedling diameter, number of leaves/plant, leaf area, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, shoot/root ratio and seedling biomass. The data were statistically analyzed for each character in a randomized block design. Analysis of variance was done as per **Panse and Sukhatme (1978)** and genetic divergence by using non-hierarchical Euclidean cluster analysis **(Spark, 1973).**

Result and Discussion

Genetic diversity in plant species is a gift to mankind as it forms the basis for selection and further improvement. The availability of statistical tool to quantitatively measure the genetic divergence between two or more populations and the relative contribution of individual character to the total divergence has permitted the tracing of evolutionary patterns in some annual species such as rice and tobacco in specific choosing parents for hybridization and general for many crop plants and a few forest tree species **(Burley and Burrows, 1972; Andrews, 1973)**. In addition to it, the basic premise of the genetic divergence is to cluster the large number of selected phenotypically superior batches into several small homogeneous groups, so as to include representative plants from each group to reduce the total number of entries in provenance/ progeny trial and seed orchard and to find out the magnitude of divergence among groups, so that the groups having wider genetic variance could be hybridized (crossed) to produce greater genetic diversity at a later period for selection. The study of relationships is based on the assumption that the difference in the characters reveals their genetic divergence.

Mean performance of 30 plus tree progeny for different morphological and biomass traits shows highest value for seedling height, no. of leaves/seedling (13.18), (5.70) for S_{30} , collar diameter (3.13) for S_{18}, Leaf area (25.17) for S_{29}, shoot fresh weight, shoot dry weight and seedling biomass (3.58), (2.51), and (3.66) for S_{27}, root fresh weight, root dry weight (1.70), (1.30) for S_{10} and shoot/root ratio (2.36) for S_{20} (Table 2).

The analysis of variance revealed the existence of significant difference among the superior tree progenies for all the traits studied, indicating the existence of genetic variability. The clustering pattern in the present study showed that 30 plus trees of *Diospyros melanoxylon* are grouped into six clusters on the basis of morphological and biomass traits under field environment (Table 3). Cluster II and IV exhibited the highest number of 8 and 7 plus tree progenies (S_1, S_2, S_3, S_8, S_{12}, S_{17}, S_{24}, and S_{30}) and (S_4, S_6, S_{18}, S_{19}, S_{20}, S_{21}, and S_{22}) respectively. Cluster I and VI comprehended six (S_5, S_7, S_9, S_{11}, S_{14}, and S_{16}) and five (S_{13}, S_{23}, S_{26}, S_{27}, S_{29}) and cluster III and V exhibited one (S_{10}) and three (S_{15}, S_{25} and S_{28}) plus tree progenies. The distribution of plus tree progenies in different clusters indicates that, even though the genotypes were selected from different eco-geographic areas indicates their close affinity. The genetic makeup along with breeding system, heterogeneity, genetic drift, natural and unidirectional selection pressure must be the cause of genetic diversity among different progenies besides geographic variation to some extent. The cluster pattern proved that geographical diversity need not necessarily be related to genetic diversity. Therefore, selection of genotypes for hybridization may be made on the basis of genetic diversity rather than geographic diversity. The present result supports the findings in *Gliricidia sepium* and *Sorbus torminalis* **(Salazar, 1986; Bednorz *et. al.*, 2006).**

The generalized inter-cluster distance (264.22) was observed highest between cluster IV and III which was followed by VI and III (243.48) (Table 4). The minimum inter-cluster distance was observed between cluster V and IV (51.12). The maximum intra cluster was found in cluster II (32.92). The inter-cluster distance was as high as IV and III (264.22) which indicated presence of higher order of divergence among the progeny of plus trees under field environment. The clustering pattern in this study revealed that superior tree progenies from different geographic region were grouped together in a cluster and vice-versa suggesting that geographical diversity did not necessarily represent the genetic diversity. **Anand *et. al.*, (2005)** reported that the factors other than geographical diversity might be responsible for their genetic uniformity. The trees/provenances that originated in one region had been distributed into different clusters indicated the trees with same geographic origin could have undergone change for different characters under selection. The present result also supports the findings in *Bauhinia variegata* and *Morus alba* that the no eco-geographical link with cluster pattern of the genotypes and their progenies **(Wani and Chauhan 2007; Thakur and Chauhan, 2008).**

Comparison of cluster means for eleven characters indicated that different characters showed considerable difference between the clusters (Table 5). Maximum value for seedling height (12.60), no. of leaves/seedling (5.53), leaf area (22.65) shoot fresh weight (3.337), root fresh weight (1.54), shoot dry weight (2.29), root dry weight (1.17), shoot/root ratio (2.15) and seedling biomass (3.47) recorded maximum value for cluster II. Whereas, cluster VI recorded maximum mean values for collar diameter (2.915). The contribution of different traits towards diversity is given in Table 5. Seedling height contributed the maximum (43.13 %) followed by leaf area (28.07 %) collar diameter (16.23 %), number of leaves/seedling (5.26 %), shoot fresh weight (3.18 %), root fresh weight (2.16 %), shoot dry weight (0.98), root dry weight (0.44), seedling biomass (0.33) and the minimum contribution was recorded for shoot/root ratio (0.86).

Heritability provides a measure of genetic variation upon which all the possibilities of changing the genetic composition of the species depend. In other words knowledge of its magnitude gives an idea about the scope for affecting genetic improvement through selection etc. while genetic gains gives relative measurement of change produced by selection in mean genetic level of the species.

The estimates of genetic parameters (Table 6) showed that shoot fresh weight had maximum PCV (45.08%) followed by collar diameter (29.95 %) and root dry weight (19.78 %). The highest GCV was exhibited by leaf area (15.62 %) followed by root dry weight (13.98 %) and collar diameter (10.55%). Estimates of heritability (broad sense) were high for leaf area (0.67 %), shoot/root ratio (0.56 %) and root dry weight (0.50 %). Genetic gain was highest for root dry weight (19.53 %), followed by shoot/root ratio (6.21 %) and collar diameter (5.31 %). Genetic advance was recorded maximum for (26.38) followed by root dry weight (20.35) and collar diameter (13.16). PCV values were higher than the correspondence GCV values for most of the traits indicate that they are much influenced by the environment. The influence of environment on the expression of morphology and biomass traits was reported in *Pinus roxburghii* **(Roy *et. al.*, 2004)** and in *Alnus nitida* **(Thakur *et. al.*, 2000).** Therefore environmental influence should be considered to select these characters towards their further improvement. Moderate to low heritability and genetic advance in shoot fresh weight, shoot dry weight, root fresh weight and root dry weight indicate that heritability in these traits is mainly due to non additive gene interaction in the inheritance of these traits and improvements could be brought through heterosis breeding. The present result substantiates the findings in *Pinus roxburghii,* and in *Prosopis cineraria* (**Roy. et. al., 2004; Manga and Sen, 2000).**

Table1: Passport details of morphological observations and their relevant information for 30 plus trees of *Diospyros melanoxylon.*

State	Notation of plus trees	District	Latitude*	Longitude *	Tree ht. (m)	Tree dia (m)	No. of seeds /fruit	Seeds length (cm)	Seeds dia (cm)
Madhya Pradesh	S_1	Rewa	24º50' N	81 º 30'E	18.0	1.5	4.4	1.42	0.76
	S_2	Jabalpur	23º13' N	79 º 67'E	16.0	1.8	4.2	1.38	0.68
	S_3	Umaria	23º69' N	80 º 96'E	17.5	2.0	5.0	1.46	0.76
	S_4	Dindori	22º94'N	81 º 07'E	18.0	1.5	4	1.22	0.78
	S_5	Sagar	24º04'N	78 º 95'E	19.0	1.6	4.4	1.32	0.72
	S_6	Damoh	23º84'N	79 º 45'E	16.5	1.9	4.2	1.28	0.62
	S_7	Hoshangabad	22º40'N	78 º 20 'E	16.0	2.2	4.8	1.28	0.68
	S_8	Bhopal	23º34'N	76 º 34'E	15.0	2	4.2	1.35	0.72
	S_9	Shivpuri	25º17'N	78 º 18'E	17.5	1.5	3.2	1.25	0.75
	S_{10}	Narsimhapur	23º40'N	79 º 55'E	18.5	1.8	4.0	1.44	0.72
Chhattisgarh	S_{11}	Raigarh	22 º 29'N	83 º 65'E	16.0	2.5	3.0	1.76	0.64
	S_{12}	Bijapur	16 º 82'N	75 º 72'E	15.5	2.2	3.4	1.52	0.68
	S_{13}	Korba	22 º 35'N	82 º 68'E	18.0	1.5	4.2	1.45	0.62
	S_{14}	Surguja	23 º 11'N	83 º 19'E	17.5	2.1	3.0	1.62	0.72
	S_{15}	Bilaspur	22 º 10'N	82 º 07'E	18.5	2.0	3.2	1.58	0.72
	S_{16}	Kondagaon	19 º 57'N	81 º 66'E	15.0	1.8	3.8	1.54	0.68
	S_{17}	Surajpur	23 º 22'N	82 º 84'E	16.5	2.2	3.4	1.48	0.64
	S_{18}	Dhamtari	20 º 42'N	81 º 33'E	15.0	1.5	3.8	1.45	0.76
	S_{19}	Baster	21 º 27'N	81 º 89'E	16.0	1.9	3.2	1.58	0.68
	S_{20}	Raipur	21 º 56'N	81 º 69'E	17.5	2.0	3.6	1.48	0.78
Jharkhand	S_{21}	Simdega	22 º 64'N	84 º 86'E	20.0	2.5	4.6	1.56	0.72
	S_{22}	Gumla	23 º 00'N	84 º 50'E	19.5	2.0	4.2	1.52	0.68
	S_{23}	Palamu	24 º 03'N	84 º 47'E	18.0	1.5	4.6	1.48	0.62
	S_{24}	Hazaribagh	24 º 20'N	85 º 35'E	18.5	2.2	3.8	1.55	0.72
	S_{25}	Ramgarh	23 º 60'N	85 º 52'E	19.0	2.0	4.2	1.45	0.74
	S_{26}	Giridih	24 º 18'N	86 º 30'E	20.0	2.5	4.4	1.62	0.68
	S_{27}	Koderma	24 º 47'N	85 º 60'E	16.0	2.2	4.8	1.54	0.72
	S_{28}	Latehar	23 º 73'N	84 º 47'E	17.5	2.0	5.0	1.52	0.74
	S_{29}	Garhwa	24 º 10'N	83 º 52'E	16.5	2.5	4.6	1.55	0.74
	S_{30}	Singhbhum	23 º 35'N	85 º 75'E	18.5	1.5	5.0	1.58	0.64

*** Encarta U.S. geological survey**

Table 2: Mean performance of 30 Plus trees progenies of *Diospyros melanoxylon*

Traits Sites	Seedling Height (cm)	Collar Diameter (mm)	No. of leaves / Seedling	Leaf Area (cm^2)	Shoot Fresh Weight (g)	Root Fresh Weight (g)	Shoot Dry Weight (g)	Root Dry Weight (g)	Shoot/ Root Ratio	Seedling Biomass (g)
S_1	10.08	2.33	4.93	12.96	2.32	1.15	1.67	0.75	2.02	2.43

S_{2}	10.31	2.21	4.75	15.57	2.28	1.21	1.60	0.74	1.89	2.35
S_{3}	11.58	2.20	4.80	16.44	2.45	1.20	1.72	0.75	2.04	2.47
S_{4}	12.37	2.06	5.20	17.63	2.59	1.15	1.84	0.78	2.23	2.62
S_{5}	11.18	2.22	5.13	15.33	2.56	1.28	1.80	0.93	1.98	2.73
S_{6}	10.80	2.34	4.86	15.06	2.60	1.18	1.78	0.82	2.20	2.62
S_{7}	10.68	2.14	4.90	16.87	2.70	1.33	1.83	0.98	2.02	2.82
S_{8}	11.13	2.22	5.15	17.57	2.92	1.55	1.96	1.14	1.87	3.11
S_{9}	10.90	2.11	4.91	14.58	2.88	1.49	1.89	1.11	1.91	3.02
S_{10}	12.79	2.73	5.50	21.00	3.33	1.70	2.18	1.30	1.97	3.49
S_{11}	10.66	2.35	5.13	14.65	2.61	1.46	1.73	1.07	1.78	2.81
S_{12}	12.06	2.55	5.26	18.06	3.22	1.60	2.18	1.20	2.00	3.39
S_{13}	12.16	2.75	5.20	17.80	3.00	1.55	2.08	1.17	1.92	3.25
S_{14}	11.26	2.49	4.86	15.40	2.76	1.42	1.85	1.06	1.93	2.92
S_{15}	12.12	2.45	4.86	18.63	2.76	1.37	1.86	1.03	2.00	2.90
S_{16}	12.22	2.35	5.06	17.93	3.13	1.51	2.12	1.14	2.06	3.26
S_{17}	11.37	2.43	5.20	19.32	2.78	1.40	1.81	1.04	1.96	2.86
S_{18}	11.86	3.13	5.23	20.70	3.09	1.41	2.13	1.08	2.18	3.21
S_{19}	12.18	2.92	5.33	19.57	3.48	1.50	2.39	1.18	2.31	3.57
S_{20}	11.28	2.90	5.00	19.23	3.09	1.30	2.15	1.03	2.36	3.18
S_{21}	11.30	2.82	4.80	18.00	3.03	1.40	2.12	1.13	2.16	3.25
S_{22}	12.12	2.79	5.00	21.74	3.24	1.39	2.27	1.07	2.30	3.34
S_{23}	11.78	2.68	5.36	21.75	3.05	1.45	2.13	1.15	2.11	3.29
S_{24}	11.73	2.46	4.86	18.19	3.12	1.55	2.13	1.18	1.98	3.31
S_{25}	8.76	1.51	4.00	13.90	2.23	1.05	1.46	0.62	1.41	2.08
S_{26}	11.67	2.74	5.43	20.03	3.39	1.52	2.32	1.15	2.22	3.47
S_{27}	13.03	2.81	5.60	23.87	3.58	1.55	2.51	1.14	2.31	3.66
S_{28}	11.63	2.48	5.33	20.76	2.98	1.49	2.00	1.12	1.98	3.12
S_{29}	13.18	2.37	5.60	25.17	3.32	1.48	2.34	1.13	2.22	3.47
S_{30}	13.18	2.61	5.70	24.12	3.33	1.56	2.28	1.18	2.11	3.47
Mean	**11.58**	**2.47**	**5.10**	**18.39**	**2.93**	**1.41**	**2.00**	**1.04**	**2.05**	**3.05**
SE (±)	0.58	0.19	0.24	1.15	0.28	0.11	0.18	0.08	0.08	0.25
C.D .5%	1.64	0.56	0.68	3.28	0.79	0.32	0.53	0.23	0.24	0.73

Table 3: Distribution of 30 plus tree progenies into different clusters.

Cluster	Total number of tree in each cluster	Notation of tree
I	6	S_{5}, S_{7}, S_{9}, S_{11}, S_{14}, S_{16}
II	8	S_{1}, S_{2}, S_{3}, S_{8}, S_{12} , S_{17} , S_{24}, S_{30}
III	1	S_{10}
IV	7	S_{4} , S_{6} , S_{18}, S_{19}, S_{20}, S_{21}, S_{22}
V	3	S_{15}, S_{25}, S_{28}
VI	5	S_{13}, S_{23}, S_{26}, S_{27}, S_{29}

Table 4: Intra and Inter cluster distances of 30 plus trees of *Diospyros melanoxylon*.

Cluster	**I**	**II**	**III**	**IV**	**V**	**VI**
Parameters ⟶						
I	**29.90**	55.26	150.99	70.62	66.01	61.01
II		**32.92**	159.81	138.51	113.44	86.25
III			**0.00**	264.22	184.70	243.48
IV				**25.26**	51.12	57.94
V					**27.28**	57.85
VI						**24.74**

Underlined figures represent intra cluster distance

Table 5: Mean values of various traits in different clusters

Clusters→ **Traits ↓**	I	II	III	IV	V	VI	**Percent Contribution**
Seedling Height	11.18	12.60	8.76	11.77	11.41	11.75	43.13
Collar Diameter	2.34	2.65	1.51	2.14	2.39	2.91	16.23
No. of leaves / Seedling	4.97	5.53	4.00	5.16	5.03	5.07	5.26
Leaf Area	16.70	22.65	13.90	16.48	17.02	19.85	28.07
Shoot Fresh Weight	2.67	3.33	2.23	2.57	2.89	3.18	3.18
Root Fresh Weight	1.33	1.54	1.05	1.22	1.47	1.40	2.16
Shoot Dry Weight	1.82	2.29	1.46	1.82	1.95	2.21	0.98
Root Dry Weight	0.93	1.17	0.62	0.85	1.10	1.10	0.44
Shoot/ Root Ratio	2.00	2.15	1.41	2.11	1.95	2.26	0.23
Seedling Biomass	2.76	3.47	2.08	2.67	3.06	3.31	0.33

Traits	**Mean**	**Coefficient of Variability**		**heritability (Broad Sense)**	**Genetic gain**	**Genetic Advance**
		Phenotypic (PCV)	**Genotypic (GCV)**	**(h^2)**	**(G.G)**	**(G.A)**
Seedling Height	11.58	10.90	6.56	0.36	0.70	8.13
Collar Diameter	2.48	29.95	10.55	0.37	5.31	13.16
No. of leaves / Seedling	5.10	9.37	4.53	0.23	0.88	4.52
Leaf Area	18.40	17.41	15.62	0.67	1.43	26.38
Shoot Fresh Weight	2.93	45.08	7.89	0.18	2.36	6.94
Root Fresh Weight	1.41	16.13	7.61	0.22	5.24	7.39
Shoot Dry Weight	2.01	18.49	8.57	0.21	4.07	8.19
Root Dry Weight	1.04	19.78	13.98	0.50	19.53	20.35
Shoot/ Root Ratio	2.05	11.13	8.30	0.56	6.21	12.74
Seedling Biomass	3.05	17.81	10.03	0.32	3.81	11.64

Table 6: Progeny variability parameter for germination characters of different plus trees of *Diospyros melanoxylon*

Conclusion

Thirty plus trees progenies were grouped into five clusters on the basis of morphological and biomass traits in which Cluster II exhibited the highest number and maximum intra cluster distance indicating that progenies in this cluster were more diverse than the other clusters. The maximum inter cluster D^2 value was obtained between cluster IV and III. The clustering pattern in this study revealed that geographical diversity did not necessarily represent the genetic diversity. Seedling height followed by leaf area contributed the maximum towards divergence. PCV was higher than the corresponding GCV which revealed that the traits were sensitive to environmental fluctuations. Hybridization between the more diverse genotypes of *Diospyros melanoxylon* can produce genotypes with high heterotic vigor. Considering the heritability estimates and genetic gain from our present study, it can be concluded that leaf area and seedling height showed better results, which indicated additive gene action on these two traits. Therefore these two traits would give better results for improvement of *Diospyros melanoxylon* by simple selection.

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