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Assessment of genetic variation in accessions of sesame (*Sesamum indicum* L.) and its crosses by seed protein electrophoresis

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Abstract

Seed storage proteins of seventeen accessions and seven crosses of sesame were analysed using polyacrylamide gel electrophoresis with a view to assessvariation. A total of 19 protein polypeptide bands were detected with relative migration of 0.13 to 0.87. The maximum number of protein bands (12) was recorded in EVAx69B-882 and the minimum (6) in 69B-882xPACH, 69B-882xEVA, C-K21xPACHcrosses and E-8 accession. Band number 1 and 13 were exclusive to 65-8B while 5 to 69B-882xC-K21. Each genotype was distinct from the other, but certain bands were shared by several genotypes. Principal component analysis of protein data revealed the first three principal components accounting for 57.08% of the total variability. The dendrogram generated from cluster analysis grouped the accessions and the crossesinto four distinct clusters. This study reveals moderate genetic variability among the genotypes from various sources and suggests possibilities for improvementthrough breeding.

Keywords: SDS-PAGE, polypeptide bands, genetic variability, cluster analysis, crosses, Sesame

1. Introduction

In Africa, Sesame production accounts for about 15% of the world's total output with Nigeria, Sudan and Uganda being the key growers [11]. Nigeria is one of the largest exporters of sesame seeds in the world with the country occupying the 6th position in 2000 as the major producer [14]. This position was maintained until 2007, when Nigeria dropped to the 7th position as the major producer [14]. The crop, often referred to as beniseed is widely used and very popular in parts of the central, North Western and North Eastern Zones where it is usually grown [13]. Sesame seed is highly nutritive and contains 50-60% oil and 25% protein with antioxidants and lignans such as sesamolin and sesamin. The lignan contents have beneficial physiological effects in animals and human health [5]. Sesame meal is notable for its high protein content which is rich in methionine and tryptophan, amino acids that are rarely found in other sources of vegetable protein such as soya. Composition of fatty acids in sesame oil is variable between different cultivars [6,9]. The high stability coupled with a nutritionally acceptable fatty acid composition contributes significantly to the excellent oil quality, making it a highly- valued edible oil. These important characteristics stimulated the interest of the researchers in biochemical analysis to identify the accessions with high nutritive values for further improvement using advanced technologies.

In Nigeria, large collections of sesame germplasm are conserved at National Cereal Research Institute, Badeji and some other outlets across the country. Breeding efforts so far has concentrated on characterization and cataloguing of germplasm collections using only morpho-agronomic traits. This however is not enough for a plant like sesame where in most cases there are absences of reliable

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morphological markers. In addition, the use of cytological techniques in the characterization of sesame species has been reported to be very difficult because of the small size of their chromosomes [28]. Therefore, a more useful and reliable tool is required to infer genetic relationships among different collections of sesame in order to facilitate the current breeding efforts for seed yield and oil quality improvement.

Characterization of germplasm using biochemical fingerprinting has got special attention due to its increased application to crop improvement and selection of desirable genotypes for breeding programs [16]. Analysis of protein and isozymes is a tool for supplementing the evidence obtained by comparative morphology, breeding experiments and cytological analysis [4]. The seed storage protein analysis helps in identification and characterization of diversity in crop varieties, cultivars and their wild relatives, thus also providing information on phylogenetic relationship of the accessions [30,37]. It isconsidered to be a practical and reliable method for species identification since seed storage proteins are highly independent of environmental fluctuations [20, 21, 22, 30].

The banding patterns produced by seed protein electrophoresis have been used to effectively characterize cultivars of *Brassicanapus* [23], Chickpea [24], *Vicia* species [32], *Brassica* species [27, 35, 38], lines of *Capsicumannuum* [4], genotypes of Chili Peppers [3], Soybean lines [12], accessions of *Lathyrussativus* [33] and landraces of sesame [17]. Despite many reports of SDS-PAGE seed storage protein profiles in a number of crops, its application to sesame plant is very limited and inadequate. Therefore the present study is undertaken to assess the genetic variability in seventeen accessions of sesameand seven crosses for further improvement in breeding programs.

2. Materials and Methods

Twenty-four sesame seeds consisting of seventeen accessions from different sourcesin Nigeria and seven crosses developed at the Department of Pure and Applied Biology, LadokeAkintola University of Technology, Ogbomoso, Nigeria,were used for SDS-PAGE (Table 1.). For the extraction of protein for electrophoresis, 1g each of the seeds of the accessions and hybrid lines was grinded to fine powder with the aid of mortar and pestle. Being an oil seed, the fine powder was first defatted with n-hexane and later homogenized in 0.9% phosphate-buffered saline for 10min using vortex. The homogenate was centrifuged at 15,000rpm for 15min before the clear supernatant was transferred into 1.5ml capacity eppendorf tube and stored at -70^{o}C in deep freezer. To 10µl of each protein extract from each sample was added 10µl of sample buffer (0.5M Tris-Hcl/pH 6.8, 10% SDS, 10% glycerol, 1% (w/v) bromophenol blue and 7.5% 2-mercaptotoethanol) and were boiled at 95^{o}C for 5min.

Seed proteins were separated by carrying out electrophoresis in the discontinuous buffer system through vertical slab type SDS-PAGE [25]. Four and twelve percent stacking and resolving gels were used respectively in the Biorad Mini Protean II cell system with power pack (Model 200/2.0) powered at 150V for 45min.When the tracking dye was at the bottom of the gel,the system was disconnected, the gels were carefully removed and stained in Coomassie Brilliant Blue solution (0.1g Coomassie Brilliant Blue (Sigma R 250), 45ml methanol, 10ml acetic acid and 45ml distilled water) for 10hrs. This was followed with overnight distaining in the same solution without the Coomassie Brilliant Blue on a shaker until the gels became clearer. They were then removed, scanned and saved on the computer system for analysis.

Data Analysis. The gels were observed for monomorphic and polymorphic protein bands on the basis of which banding electrophoregrams were prepared.The Relative Migration (RM) [29] of each band was calculated as follows;

RM = Distance migrated by the protein band from origin (cm) / Distance migrated by tracking dye (cm)

Polypeptide bands were scored for presence (1) or absence (0) and entered in a binary data matrix for use in cluster analysis. Cluster analysis was performed on SPSS computer software (version 15).

3. Results

On the basis of the relative mobility of seed proteins on the gel, 19 bands were detected and were used to explore genetic diversity (Fig.1., Tab. 2). Maximum number of bands (12) was found in EVAx69B-882 hybrid while minimum number (6) occurred in 69B-882xPACH75, 69B-882xEVA, C-K2xPACH crosses and E-8 accession. The bands relative migration distance varied from 0.13 to 0.87(Table 1.).Factoranalysis revealed that the first threeprincipal components accounted for 57.08% of the total variability among the accessions and the

crosses (Tab 3.). The importance and relationship between variables within a component are determined by the magnitude and direction of a factor loading within a PC. Principal component 1 accounted for 29.23% of the variation in protein bands with high positive loading for protein bands 2, 4, 6, 8, 16, high negative loading for bands 7, 10, 11, 18, 19 but low negative loading for bands 12 and 17. An additional 16.46 of the total variability was contributed by the principal component 2 with high positive loading for bands 1, 13, 15, high negative loading for bands 3 and 14. Principal component 3further accounted for 11.39%of the total variability with high positive loading for protein band9 and low negative loading for band 5. However, none of the protein bands was redundant.

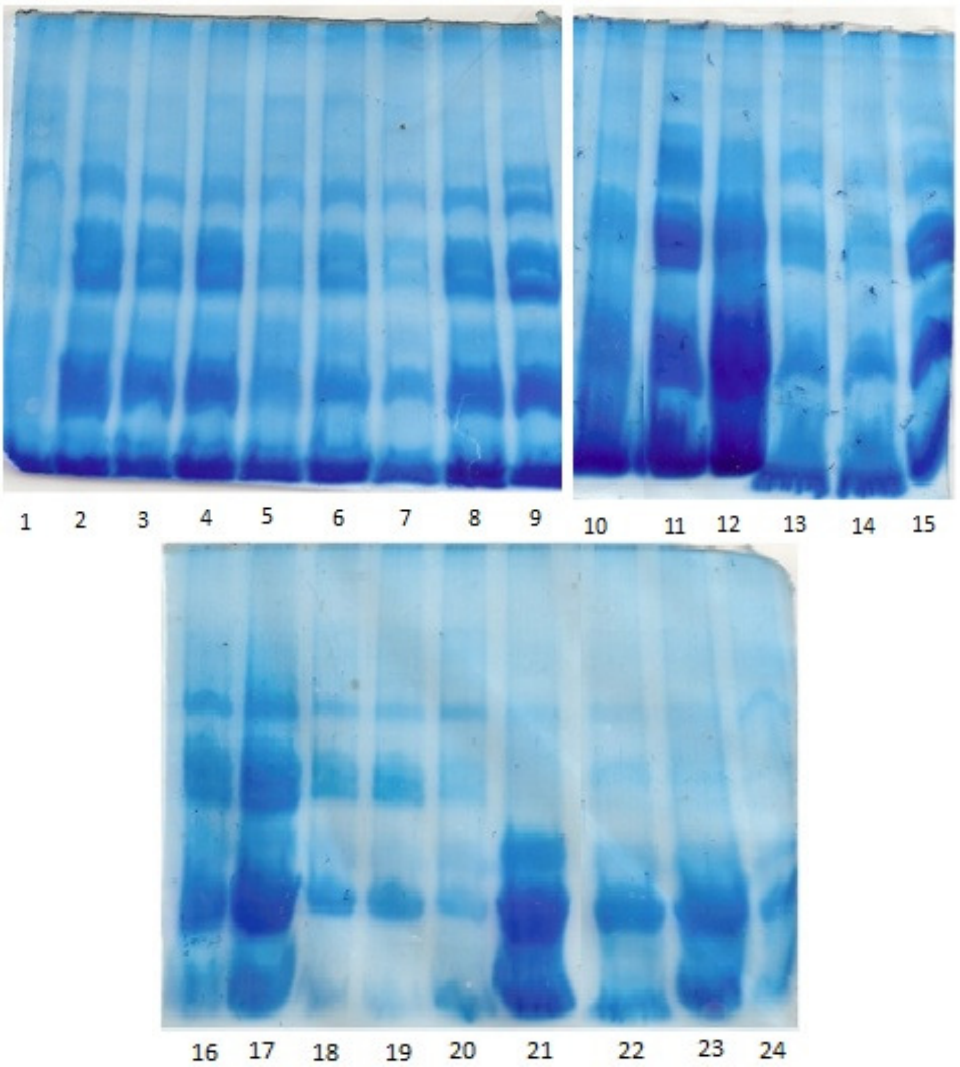

Figure 1. Seed protein banding pattern of the seventeen accessions and seven crosses of sesame studied

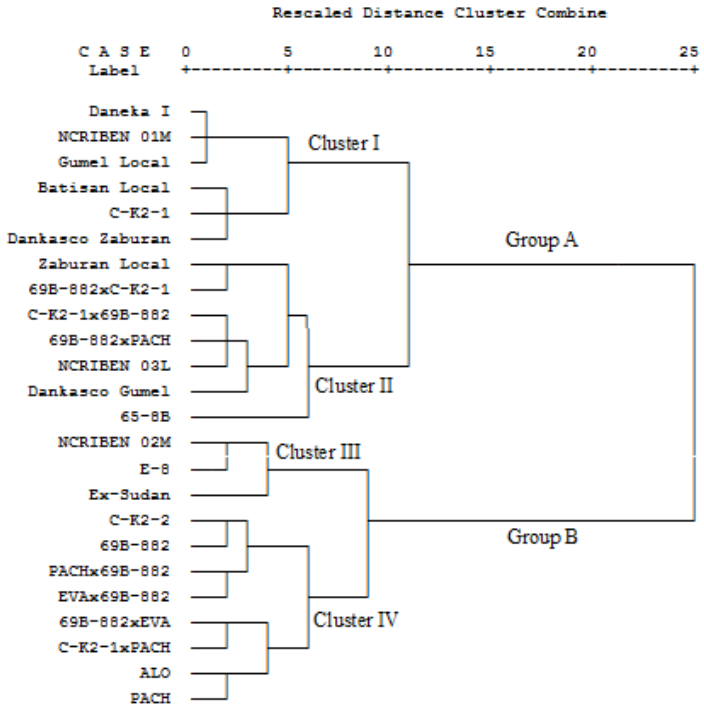

Figure 2. Dendrogram of seventeen accessions and seven crosses of sesame studied by Ward's method

Table 1. Name of the seventeen accessions and seven crosses of sesame studied, sources, number of protein bands and relative mobility range

S/No	Accession/ hybrid lines	Source/ Origin	No of protein bands	Relative migration range
1	DankascoGumel	Jigawa State	8	0.18 – 0.67
2	Daneka I	Katsina State	10	0.18 – 0.83
3	NCRI BEN 01M	NCRI Badegi	10	0.18 – 0.83
4	Gumel Local	Jigawa State	11	0.18 – 0.83
5	Batsari Local	Katsina State	10	0.18 – 0.8
6	Dankascozaburan	Jigawa State	10	0.18 – 0.80
7	C-K2-1	*NCRI, Badeji	8	0.18 – 0.80
8	C-K2-2	NCRI, Badeji	8	0.35 – 0.83
9	69B-882	NCRI, Badeji	8	0.35 – 0,83
10	NCRI 03L	NCRI, badeji	8	0.18 – 0.76
11	Zaburan Local	Jigawa State	7	0.18 – 0.76
12	65-8B	NCRI, Badeji	11	0.13 – 0.80
13	69B-882xC-K2-1	LAUTECH, Ogbomoso	7	0.18 – 0.76
14	C-K2-1x69B-882	LAUTECH, Ogbomoso	8	0.29 – 0.76

Table 1 (continued)

15	69B-882xPACH	LAUTECH, Ogbomoso	6	0.29 – 0.76
16	PACHx69B-882	LAUTECH, Ogbomoso	9	0.35 – 0.87
17	EVAx69B-882	LAUTECH, Ogbomoso	6	0.29 – 0.87
18	69B-882xEVA	LAUTECH, Ogbomoso	6	0.38 – 0.87
19	C-K2-1xPACH	LAUTECH, Ogbomoso	7	0.38 – 0.87
20	ALO	LAUTECH, Ogbomoso	10	0.38 – 0.83
21	PACH	NCRI, Badegi	9	0.29 – 0.87
22	NCRI 02M	NCRI, Badegi	9	0.29 – 0.87
23	Ex-Sudan	NCRI Badeggi	6	0.23 – 0.87
24	E-8	NCRI Badeggi	12	0.29 – 0. 83

*National Cereal Research Institute, Badegi, Nigeria

Table 2. Distribution of protein bands in the seventeen accessions and seven crosses of sesame studied

Bands	RM	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	0.13	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
2	0.18	1	1	1	1	1	1	1	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
3	0.23	1	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
4	0.28	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	1	0	0	0	1	1	1	1
5	0.32	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
6	0.35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1
7	0.38	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	1	1	1	1	1	1	1	0	0
8	0.42	1	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	1	0
9	0.47	1	1	1	1	1	1	1	1	0	1	0	1	0	1	0	1	1	0	0	0	1	0	0	0
10	0.52	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1
11	0.57	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0
12	0.60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0
13	0.63	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
14	0.67	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	0	1	0	0	1	1	0	0	0
15	0.72	0	0	0	0	0	1	0	0	0	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1
16	0.75	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	0
17	0.80	0	0	0	0	1	1	1	1	1	0	0	1	0	0	0	1	1	0	1	1	1	1	0	1
18	0.83	0	1	1	1	0	0	0	1	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
19	0.87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	1	1	0

Table 3. Eigenvectors and percentage explained variation by the first five principal components of protein band profile of the seventeen accessions and crosses of sesame studied

Eigenvectors					
Protein Bands	Prin 1	Prin 2	Prin 3	Prin 4	Prin 5
Band 1	0.272	0.753	0.552	-0.047	0.011
Band 2	0.757	-0.187	0.263	-0.200	0.361
Band 3	0.451	-0.557	0.180	0.250	0.273
Band 4	0.694	-0.006	-0.098	0.341	0.364
Band 5	0.272	0.113	-0.457	-0.595	0.315
Band 6	0.667	-0.153	0.119	0.245	0.149
Band 7	-0.714	0.375	0.297	-0.146	0.155
Band 8	0.511	0.379	-0.084	0.464	-0.439
Band 9	0.314	-0.283	0.676	0.116	0.058
Band 10	-0.584	-0.515	0.123	0.418	-0.181
Band 11	-0.594	-0.473	0.455	-0.336	0.032
Band 12	-0.492	0.173	-0.134	0.275	0.565
Band 13	0.272	0.753	0.552	-0.047	0.011
Band 14	0.350	-0.535	-0.022	-0.392	-0.187
Band 15	0.245	0.549	-0.413	-0.090	-0.014
Band 16	0.523	-0.153	0.251	-0.054	0.017
Band 17	-0.488	0.217	0.416	-0.148	-0.032
Band 18	-0.792	-0.150	0.071	0.041	0.078
Band 19	-0.707	0.221	-0.123	0.318	0.319
Eigenvalue	5.554	3.128	2.164	1.541	1.230
Individual percentage	29.233	16.462	11.389	8.110	6.474
Cumulative percentage	29.233	45.696	57.084	65.194	71.668

Table 4. Clusters of the seventeen accessions and crosses of sesame studied

Groups	Clusters	Frequency	Accessions/Hybrid lines
Group A	Cluster I	6	Daneka I, NCRIBEN 01M, Gumel local, Batsari local, C-K2-1, Dankascozaburan
	Cluster II	7	Zaburan local, 69B-882 x C-K2-1, C-K2-1 x 69B-882, 69B-882 x PACH, NCRIBEN 03L, DankascoGumel, 65-8B
Group B	Cluster III	3	NCRIBEN 02M, E-8, Ex-Sudan
	Cluster IV	8	C-K2-2, 69B-882, PACH x 69B-882, EVA x 69B-882, 69B-882 x EVA, C-K21 x PACH, ALO and PACH

Dendrogram of total seed proteinsusing Ward's method divided the accessions and the crosses into two main groups (A and B), each consisting of two clusters (Fig 2; Tab 4.). Cluster1 consisted of two accessions of Jigawa origin (Gumel local and DankascoZaburan), two of Katsina origin (Daneka and Batsari local), and two of NCRI Badeji (NCRIBEN 01M and C-K21). In the cluster II were two other accessions from Jigawa (Zaburan local and DankascoGumel), two from NCRI (NCRIBEN 03L and 65-8B) and three crosses from Ogbomoso (69B-882xC-K21, C-K21x69B-882 and 69B-882xPACH). The three accessions in clusterIII were from NCRI, Badegi(NCRIBEN 02L, E-8 and Ex-Sudan), while the four remaining crosses and one accession from Ogbomoso occurred in cluster IV alongside three other accessions from NCRI Badegi (C-K22, 69B-882 and PACH).

4. Discussion

Protein types and their diversity varied among a variety of crop species, which may assist the early detection of species at seed level and to acquire information on clarity of genetic assets [31]. The results of the sesame seed protein analysis indicate differential banding pattern for different accessions and crosses, but the overall degree of variation is relatively moderate. This finding is supported by Fazal et al. (2012) [17] and Ghafoor et al. (2003) [19] who reported a limited level of intra specific variation for seed protein among sesame germplasm and chickpea respectively. In a related work using SDS-PAGE, Fufa et al. (2005) [18] opined that the genetic estimates based on seed protein were lowest because they were the major determinants of end-use quality, which is a highly selected trait. However, this is contrary to the report of Nisar et al. (2007) [30] that a high level of intra specific variation for seed protein was found among local and exotic chickpea germplasm. Disparity in these results was attributed to differences in gene pools from both exotic and local resources employed in the investigation [17].

There are differences in the density of the common major bands. The major bands show similar banding pattern in some of the accessions and hybrids but diversity based on the minor bands were available in most of the accessions and hybrids studied. According to Ali et al. (2007) [2], the equality in major bands among a variety of accessions specifies that the genes codding these proteins are preserved. This report is further corroborated by Mohammad et al. (2007) [26].

Cluster analysis based on SDS-PAGE analysis of protein pepetides were more reliable where most of the accessions of each group were collected from the same geographic location [33]. This is partly true for the present finding as all accessions from Jigawa and Katsina were grouped together with few accessions from NCRI and crosses from Ogbomoso, suggesting a sort of correlation between SDS-PAGE data and geographic origin. According to Stoyanova and Boller (2010) [36], large variations and overlap within accessions probably make it difficult to create a relationship between genotypes and their origin. However, the observed overlap between most accessions from NCRI and Hybrids developed at Ogbomoso, points to the possibility of the existence of common genetic background between NCRI accessions and crosses analyzed. This is not unexpected as most of the hybrids were developed largely from materials sourced from NCRI in Nigeria being the only Institute that has the research mandate for sesame germplasm conservation and improvement.

The clustering together of some male parents and their crosses indicates the successof the crosses employed in the study. Especially in the case of69B-882 (male parent) and crosses PACHx69B-882 and EVAx69B-882, pointing to the usefulness of seed protein SDS-PAGE in the assessment of genetic relatedness between parents and their crosses. Ali et al. (2008) [1] reported higher degree of similarity between male parent and offspring compared to female parent and offspring in cotton using RAPD markers. In the present study, some crosses clustered away from their parents,which may be ascribed to the interplay of the factors such as recombination forces, deletion, mutation or random segregation of chromosomes at meiosis during the process of hybrid formation.

Principal component analysis of seed protein dataindicated that the first three principal components comprise 57.08% of total variability in the sesame samples analyzed. Bands number 1, 2, 7, 13, 18 and 19 which are highly correlated with these principal components (>0.70) are the most variable bands among the accessions and crosses of sesame studied. According to Crawford (1990) [10], seed storage proteins are coded by genes, therefore the observed variation in protein bands may be indicative of

genotypic differences among the sesame samples. In a related work on *Hyoscyamus* species [34], variation in protein bands was attributed to genomic changes taken place during the species diversification. The observed moderate variability is at consonance with the previous oil and fatty acid profile [7] and seed physical dimensions study [8]. However, the degree of differences in variability may be associated with the differences in tool used and genetic background of the sesame collections investigated.

5. Conclusion

The results of the present investigation reveal moderate genetic base for sesame collection studied. It shows relationship between the sesame samples and geographical origin and also suggests common genetic background between crosses and the source of the parent genotypes. The present study can therefore be employed in the selection of genotypes from various sources to form a wide gene pool with broad genetic base on which future breeding programme could be hinged.

Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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