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Morphological Characterisation and Variability Analysis of African Yam Bean (*Sphenostylis stenocarpa* Hochst. ex. A. Rich) Harms

Ademola I. Aina[1,2,*], Christopher O. Ilori[1], Ukoabasi O. Ekanem[2], Olaniyi Oyatomi[2], Daniel Potter[3], Michael T. Abberton[2]

[1]Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria
[2]Genetic Resources Centre, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria
[3]Department of Plant Sciences, University of California, Davis, U.S.A

Abstract African yam bean (*Sphenostylis stenocarpa*) is a legume that produces tubers rich in protein and with potential to contribute to food and nutrition security in tropical Africa. African yam bean (AYB) is underused despite its nutritional profile. Knowledge of the genetic diversity present in AYB germplasm would be beneficial to the utilisation of its genetic resources in the improvement of the crop. This study aims to understand the genetic diversity in a collection of 50 accessions of AYB. Thirty morphological and taxonomic phenotypic traits of high descriptive value were characterized and the levels of diversity among the accessions were assessed using analysis of variance (ANOVA), Correlation, Principal Components, and Clustering analysis. The 50 accessions were grouped into four clusters of 2-17 members at a dissimilarity index of 0.7. Out of 30 morphological variables analysed, eye colour pattern was the most discriminatory and it classified the accessions in the proportion of 38% with white or grey testa with incision-like eye pattern and 62% with brown testa and varying patterns from narrow stripe to fork-like or incision-like eye pattern around the hilum. The stepwise discriminant and frequency procedures in SAS also identified other phenotypic variables with significant discriminatory attributes. Accessions with desirable agronomic characters such as lower number of days to flowering, high number of pods per plant and total seed weight per plant were also identified in this study.

Keywords African yam bean, Genetic diversity, Accessions, Phenotypic traits

1. Introduction

In sub-Saharan Africa, increasing food options through effective utilisation of neglected and underused crops will help to reduce hidden hunger, environmental and income vulnerability in the face of unpredictable weather conditions [36]. African yam bean (*Sphenostylis sternocarpa* Hochst. ex A. Rich.) Harms) is an underutilised legume with potential to address the food and nutritional security issues of tropical Africa. African yam bean (AYB) produces edible tubers and seeds and is considered the most economically important species in the genus *Sphenostylis* [27,28]. This neglected legume [29,18] has been an important traditional crop in Africa with several sociocultural uses [2]. For instance, the crop is considered a significant substitute for cowpea (*Vigna unguiculata* (L.) Walp.) during lean periods in the eastern parts of Nigeria [23]. Also, the lectin found in AYB seeds has been reported to be effective in the control of some leguminous pests [24,22].

African yam bean has a good amino acid profile, containing more lysine and methionine content than is found in pigeon pea (*Cajanus cajan* (L.) Millsp.), cowpea and Bambara groundnut [33]. Its grain and tuber contain approximately 29% and 19% crude protein, respectively [2]. In West Africa, particularly Nigeria and Ghana, only few aged farmers and old market women still hold and use some available landraces [6,30], hence genetic erosion is imminent as the few landraces may be lost with the death of the farmers. Conservation ex-situ or in situ in genebanks is the only approach to preserve the genetic resources of species producing orthodox seeds [8]. The commercial exploitation of AYB will rely on superior and improved varieties.

The continual collection and assessment of the diversity present in landraces will enhance the success of breeding for hybrid varieties. Diversity analysis of AYB has been done using morphological [4,26,2,21] and molecular characterisation [16,3,20,31].

* Corresponding author:
ainaademola4@gmail.com (Ademola I. Aina)
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The Genetic Resources Centre of the International Institute of Tropical Agriculture has been carrying out timely collection of AYB for conservation. Morphological characterisation precedes selection for genetic variability and identification of promising accessions with desirable traits of interest. This present study aims to assess the genetic diversity in 50 accessions of AYB and to identify discriminatory phenotypic variables in AYB.

2. Materials and Methods

2.1. Plant Materials and Experimental Lay Out

Fifty accessions of African yam bean (AYB) were obtained from the Genetic Resources Centre, International Institute of Tropical Agriculture (IITA), Ibadan. The passport data of the selected accessions are presented in (Table S1). Two to three seeds of each accession were sown on double row plots of 5m long each with an intra and inter-row spacing of 1m apart. Seedlings were thinned to one plant per stand after emergence. The experiment was performed during the cropping seasons of 2015/16 and 2016/17 (August to February). Three weeks after planting, the seedlings were staked and carefully twined in a clockwise manner around the stakes. Insects were controlled with a mixture of Paraeforce and Cyperdiforce at the rate of 35-60 mL in 20 litres of water every 2 weeks during the flowering period. Weeding and other field maintenance practices were performed until maturity. The experiment was laid out in a randomised complete block design and replicated three times. Data on morphological variables were taken and recorded from five representative plants of each accession on the two plots, using the IITA descriptor list for AYB [1].

2.2. Data Analysis

The Gower distances [9] for continuous, ordinal and binary variables were calculated and cluster analysis of the AYB accessions using Ward minimum variance method [34]. Analysis of variance (ANOVA) and Stepwise discriminant approach [10] were applied on the continuous and log transformed ordinal variables to determine the critical variables on the clustering groups. The step discriminant model identifies variables in a sequential manner, starting with the highest F value from an ANOVA and explores the possibility of any relationship between the observed variables. A maximum *P* value of 0.15 was employed in assigning a new variable in a set of significant variables, as recommended by [10]. Principal component analysis was calculated to determine the interrelationship among morphological variables using PRINCOMP procedure in SAS-V9 [37]. The relationship between pairs of morphological traits was calculated using Pearson's correlation coefficient [25]. ANOVA, Cluster analysis, Step Discriminant analysis was performed using CLUSTER, GLM and STEPDISC from the Statistical analysis system, SAS-V9.3 [37].

Plate 1. AYB plants growing in the field

3. Results and Discussion

Most (80%) of the accessions studied here were collected from different parts of Nigeria. The rest 20% had not available passport data. A total of 30 variables were used in descriptive statistics for the accessions studied. Twenty-two continuous variables were recorded while the eight ordinal variables were all seed and tuber related. The variables showed variation in their strength for distinguishing the 50 accessions. For instance, the pod length was about six times the length of the shorter ones (Table S1) and days to physiological maturity varied considerably from 132 to 165 days after planting.

Table 1. Stepwise discriminant analysis for continuous, log transformed ordinal and binary variables

Steps Variables	Partial R^2	F-Value
Continuous variables		
1 100-seed weight	0.5993	2.93(<.0001)
2 Seed weight per pod	0.5888	2.79(<.0001)
3 Number of seeds per pod	0.5454	2.33(<.0001)
4 Days to 50% flowering	0.4890	1.85(<.0002)
5 Seed Length	0.4795	1.77(.0004)
6 Terminal Leaf Length	0.4881	1.82(.0002)
7 Pod Length	0.4148	1.35(.0427)
8 Peduncle Length	0.4162	1.35(.0428)
Ordinal Variables		
1 Eye colour pattern	0.6004	3.07(<.0001)
Binary variables		
1 Seed cavity ridges on pods	0.4574	1.69(.0138)
2 Seed shape	0.5235	2.22(.0004)

The most discriminative ordinal variable (P ≤ 0.0001) was the eye colour pattern (Table 1) while seed shape and cavity ridges were the most discriminatory binary variables (P ≤ 0.0138). These variables in descending order of; 100-seed weight, seed weight per pod, number of seeds per pod, days to fifty per cent flowering, seed length, terminal leaf length, pod length and peduncle length were the most significant continuous variables (P ≤ 0.0427).

There were significant differences in the performances of 50 AYB accessions with reference to the significant mean square values for 19 quantitative agro-morphological traits evaluated in 2015 and 2016 cropping seasons. Highly significant differences (P<0.001) were obtained for peduncle length, pod length and seed yield and significant differences (P<0.05) for days to 50% flowering, seed length and hundred-seed weight (Table 2).

Table 2. Means, mean squares and coefficient of variation of 19 quantitative agro-morphological traits from 50 AYB accessions evaluated in 2015

Characters	Means	Mean squares	CV%
Days to 50% flowering	85.41	4.88 **	5.72
Days to phys. maturity	146.62	8.63	5.89
Leaf number per meter length	23.86	2.41	9.07
Terminal leaf length (cm)	7.82	0.55	7.07
Terminal leaf width (cm)	2.19	0.24	8.75
Peduncle length (cm)	9.55	3.26*	34.15
Pod length (cm)	21.51	3.66*	23.36
Pod weight per plant (g)	42.30	21.99	51.98
Seed weight per pod (g)	3.09	0.78	25.31
Seed weight per plant (g)	33.75	18.62	55.19
Seed length (mm)	8.06	0.47**	5.85
Seed width (mm)	6.31	0.45	7.14
Seed thickness (mm)	6.22	0.70	11.24
Number of locules per pod	14.18	2.14	15.13
Number of seeds per pod	13.82	2.28	16.50
Seed set percentage	97.44	5.87	6.02
Shelling percentage	88.96	49.47	55.60
Hundred-seed weight	22.42	2.12**	9.48
Seed yield (kg/ha)	121.91	72.22*	59.24

In 2016, the mean square values were highly significant (P<0.001) for terminal leaf width, peduncle length, pod length, seed weight per plant, seed length, number of locules per pod, number of seeds per pod, hundred-seed weight and seed yield (Table 3).

Based on low distances (high similarity) among them using all the variables considered simultaneously, the 50 AYB accessions were clustered into 4 main groups (Figure 1), at an R squared distance of 0.7 dissimilarity index. The distances between accessions spanned from 0.0003 and 0.5859. The most similar accessions phenotypically were TSs 157A and TSs 302 tied with TSs 138B and TSs 304, with the least distance of 0.0003. Cluster I (Figure 1) contained 15 accessions, in this cluster, only TSs 63A had white/grey testa with incision-like eye pattern, while 93.3% of the cluster had brown testa with dark brown incision-like pattern below and parallel the hilum (Table 4). Seed cavity ridges were conspicuous on 80% of the accessions and were mostly oval or rhomboid in shape. All the accessions in this cluster produced tubers; 12 (80%) had branched tubers while the remaining TSs 30B, TSs 333 and TSs 23C had unbranched tubers. The tubers were round (13.3%), Ovate (66.7%) or irregular (20%) in shape. Accessions in cluster II had globular (12.5%), oval (37.5%), or oblong (50%) seed shapes. Half of the accessions in this group had white/grey testa with incision-like eye pattern. Most of the accessions (62.5%) showed the presence of seed cavity ridges on pods.

Table 3. Means, mean squares and coefficient of variation of 19 quantitative agro-morphological traits from 50 AYB accessions evaluated in 2016

Characters	Means	Mean squares	CV%
Days to 50% flowering	78.07	5.19	6.65
Days to phys. maturity	146.73	8.97	6.11
Leaf number per meter length	33.86	3.41	10.07
Terminal leaf length (cm)	7.82	0.55	7.07
Terminal leaf width (cm)	3.19	0.34*	10.75
Peduncle length (cm)	9.55	3.26*	34.15
Pod length (cm)	21.71	4.66*	21.46
Pod weight per plant (g)	43.02	29.81	69.29
Seed weight per pod (g)	5.07	1.49	29.37
Seed weight per plant (g)	26.93	17.80*	66.10
Seed length (mm)	8.12	0.81*	10.02
Seed width (mm)	6.34	0.79	12.50
Seed thickness (mm)	7.09	1.20	16.99
Number of locules per pod	12.79	3.20*	25.03
Number of seeds per pod	12.22	3.18*	26.03
Seed set percentage	94.99	7.23	7.61
Shelling percentage	64.53	19.01	29.45
Hundred-seed weight	22.61	3.03*	13.42
Seed yield (kg/ha)	110.92	70.26*	63.35

Only five accessions (31.25%) produced tubers that were spindle-shaped.

Cluster III is the only group that comprises non-tuberous accessions. Seed shapes were variable from globular (11.8%), oval (23.5%), oblong (41.2%) and rhomboid (23.5%). Eleven (76.5%) of the accessions in this cluster had pronounced seed cavity ridges on their pods while TSs 22B, TSs 155, TSs 358, TSs 60B and TSs 303 had no cavity ridges. Cluster IV included the fewest accessions (TSs 59B and TSs 84A). The accessions produced non-branching ovate and spindle-shaped tubers. The pods were ridged and the seeds were oval in shape. Accessions in this cluster had brown testa with dark brown incision-like pattern below and parallel the hilum. The first principal component (Table S2) correlated mostly with quantitative and seed related traits; pod length (0.2762), pod weight per plant (0.3221), seed weight per plant (0.3162), number of seeds per pod (0.3264), seed yield (0.3218) and peduncle length (0.2644). Qualitative traits relating to both seeds and tubers contributed mainly to the loading of PC2; seed cavity ridges on pods (0.2749), eye colour pattern (0.2100), production of tuber (0.4405) and tuber shape (0.4405). Number of seeds per pod (0.2577), testa colour variegation (0.4274), basal colour of variegated seeds (0.3178) contributed positively to the loading of PC3, while seed width (-0.2974) and eye colour pattern (-0.3350) also contributed negatively. Colour variegation (0.2207), terminal leaf length (-0.2447), pod weight per plant (-0.2116) and eye colour pattern (-0.2126).

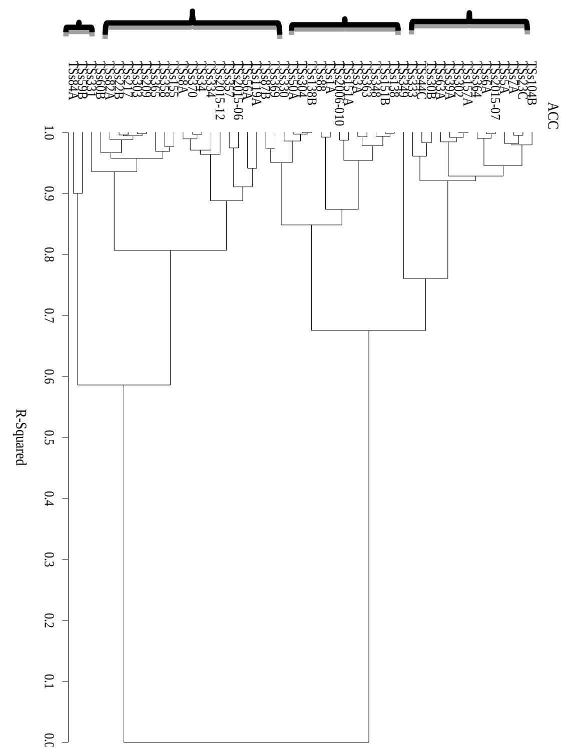

Figure 1. Dendrogram showing the clustering pattern of 50 African yam bean accessions

The fourth principal component axis had more characters contributing to its loading; days to fifty percent flowering (0.2776), seed length (0.4418), seed width (0.3198), testa colour variegation (0.2207), terminal leaf length (-0.2447), pod weight per plant (-0.2116) and eye colour pattern (-0.2126). Days to fifty percent flowering (0.2683), days to physiological maturity (0.4107), terminal leaf length (3286), terminal leaf width (0.3140) and eye colour pattern (0.2292) all contributed positively to the fifth principal component.

For the Pearson correlation coefficients, seed weight per plant was strongly and positively correlated with pod weight per plant (r=0.84). Number of pods per plant was positively and strongly correlated with pod weight per plant (r=0.72) and seed weight per plant (r=0.74). Number of seeds per pod had a strong and positive correlation with number of locules per pod (r=0.96), which was the strongest correlation found among the traits studied. These are important attributes for seed yield.

Table 4. Frequencies of ordinal and binary variables in percentage

Cluster (Number of accessions)	I(15)	II(16)	III(17)	IV(2)
Variations		Percentages (%)		
Eye colour pattern				
ECP1	-	12.5	23.5	-
ECP2	-	37.5	-	-
ECP3	6.7	50	58.8	-
ECP4	93.3	-	17.7	100
ECP5	-	-	-	-
ECP6	-	-	-	-
Seed cavity ridges on pods				
Present	80	62.5	76.5	100
Absent	20	37.5	23.5	-
Seed shape				
Round	6.7	12.5	11.8	-
Ovate	33.3	37.5	23 .5	100
Spindle	26.7	50	41.2	-
Irregular	33.3	-	23.5	-
Tuber production				
No	-	68.75	-	-
Yes	100	31.25	100	-
Tuber shape				
Round	13.3	-	-	-
Ovate	66.7	-	-	50
Spindle	-	100	-	50
Irregular	20	-	-	-
Tuber branching				
No	80	75	-	100
Yes	20	25	-	-

Legend: ECP1= Brown testa with continuous narrow black hilum around the testa, ECP2= Brown testa with dark brown fork-like eye pattern, ECP3 = White or grey testa with incision-like eye pattern, ECP4= Brown testa with dark brown incision-like pattern below and parallel the hilum, ECP5= White testa with reddish brown vase-like eye, ECP6= White testa with black vase-like eye

In this study, eye colour pattern strongly separated African yam bean accessions into two major groups. Accessions having white or grey testa with incision-like eye pattern were mostly grouped in clusters II and III, while accessions grouped in cluster I and IV had brown testa with varying degrees of incision eye pattern. Eye colour pattern is therefore an important morphological variable for the classification of AYB. It is a significant variable in discriminating peas [14]. Each of the selected AYB accessions used in this study had some superior morphological features which makes them potential parental genotypes in hybridisation for the genetic improvement of AYB. The mean square values of combined quantitative traits over a period of two years revealed significant differences among the accessions for days to 50% flowering, number of seeds per pod, seed yield and highly significant differences for seed length and hundred-seed weight. Seed-related traits have been reported to be very important in the determination of the genetic and taxonomic relationships in crops [15,11] and also in legumes [19]. The early-flowering accession TSs6A (Cluster I) flowered earlier at 75.66 days after planting compared to other accessions and can be selected as a promising parent for early maturity in AYB. Accessions TSs30B also in the same cluster flowered early with a mean number of 79 days.

The variation in days to flowering could be due to genetic factors as the mean number of days to flowering, were consistent in both years. Accessions (TSs59B, TSs84A, TSs8A, TSs358 and TSs22B) with higher values for number of leaves per meter length, number of flowers per peduncle and lower internode distance, grouped in cluster III and IV were observed to produce a high number of pods per plant and consequently higher number of seeds per plant which translates to increased yield [26] and [20] also observed this in their reports. Hence, these accessions could be good candidates for high seed yield in AYB. The length of the vines, the lush growth habit of some accessions and increased production of flowers which results in a larger number of pod and seed formation may also be responsible for high seed yield. Same observations have been recorded in cowpea and pigeon pea [26,35]. TSs50A recorded the lowest yield (47.0 kg ha^{-1}) and this could be due to poor agronomic performance of the accessions across all the traits evaluated. TSs56A had the highest weight per pod (70.6g) while TSs357 had the least (18.1g).

Four seed shapes were identified in this study; 5 (10%) accessions had round seeds, 17 (34%) had oval seeds, 19 (38%) had oblong seeds and 9 (18%) had rhomboid seeds. The correlation coefficients were high and significant for yield and yield-related traits such as number of locules per pod, number of pods per plant, number of seed per plant and weight of pods per plant which contributed to high seed percentages. Similar observations have been reported by [26,12,32]. Hence, selection for these characters could contribute to higher seed yield in African yam bean accessions. Principal component analysis (PCA) identifies plants traits that characterize the distinctness among selected genotypes [7]. It also helps in the classification of populations into distinct groups based on similarities for one or several traits and guides in the selection of parents for hybridization [5,17].

In this study, 54.31% of the total variation was explained by the first five principal components, with PC1 to PC3 accounting for 40.07% of the total variation with eigenvalues ranging from 2.55 to 5.98. The 18 agro-morphological traits were significant in detecting variation among 50 accessions of AYB. Eigenvalues greater than one are considered significant and component loadings ≥ 2.0 are considered to be important [17]. In this study, 54.31% of the total variation was explained by the first five principal components, with PC1 to PC3 accounting for 40.07% of the total variation with eigenvalues ranging from 2.55 to 5.98. The 18 agro-morphological traits were significant in detecting variation among 50 accessions of AYB.

4. Conclusions

To improve AYB yield, number of seeds per pod, number of pods per plant, weight of seeds per pod and weight of seeds per plant are important factors to be considered in breeding programmes.

Appendix

Table S1. Statistical characterisation for continuous and ordinal variables used for AYB description

Continuous variables	Min	Max	Mean	Std Dev
DFF	65	97	81.8	6.53
DPM	132	165	146.7	8.83
LNPL	15	43	33.1	4.69
TLL	5.8	11.3	8.1	0.86
TLW	2.1	4.5	3.3	0.45
IL	5.1	19.1	9.1	1.84
PL	3	5.2	4.1	0.41
PEDL	0.8	18.9	10.1	3.00
PODL	5.2	30.9	21.6	3.96
PWPP	1.3	135.5	42.7	29.11
SWPP	1.3	8.6	4.1	1.55
SWP	0.6	104.2	30.4	21.18
SL	5.4	12.7	8.1	0.77
SW	3.7	12.4	6.3	0.67
ST	4	14.4	6.7	1.11
NPP	1	30	11.4	6.10
NLPP	2	23	13.5	3.09
NSPP	2	23	13.0	3.20
SSP	60	100	96.2	6.83
SP	6.1	356.8	77.0	39.18
HSW	16	32	22.5	3.37
SDY	13.7	429.3	116.5	83.37
Ordinal Variables	Min	Max	Mean	Std Dev
SS	1	4	2.5	0.78
SCRP	0	1	0.8	0.40
ECP	1	4	2.7	1.00
TCV	0	1	0.2	0.41
BCVS	0	2	0.4	0.74
TBD	0	1	0.5	0.50
TBS	0	2	0.7	0.84
TBB	0	1	0.5	0.50

CODES	FULL MEANING
DFF	Days to fifty percent flowering
DPM	Days to physiological maturity
LNPL	Leaf length per meter
TLL	Terminal leaf length
IL	Internode length
PL	Petiole length
PEDL	Peduncle length
PODL	Pod length
PWPP	Pod weight per plant
SWPP	Seed weight per pod
SWP	Seed weight per plant
SL	Seed length
ST	Seed thickness
NPP	Number of pods per plant
NLPP	Number of locules per pod
NSPP	Number of seeds per pod
SSP	Seed set percentage
HSW	Hundred-seed weight
SDY	Seed yield
SS	Seed shape
SCRP	Seed cavity ridges per pod
ECP	Eye color pattern
TCV	Testa color variegation
BCVS	Basal color of variegated seeds
TBD	Tuber production
TBS	Tuber shape
TBB	Tuber branching

Table S2. Eigenvalues, percentage cumulative variance and Eigenvectors

	PC1	PC2	PC3	PC4	PC5
Eigenvalues	5.9885	3.4810	2.5504	2.1873	2.0866
%Proportion variance per PC-axes	0.1996	0.116	0.085	0.0729	0.0696
%Cumulative variance across PC-axes	0.1996	0.3156	0.4007	0.4736	0.5431
Morphological traits		Eigenvectors			
DTFP	-0.0027	-0.0320	0.1108	**0.2776**	**0.2683**
DPM	-0.0246	0.1083	0.0110	0.1746	**0.4107**
TLL	0.1133	-0.0248	0.0411	**-0.2447**	**0.3286**
TLW	0.0156	-0.1055	-0.0113	0.0423	**0.3140**
PDL	**0.2644**	0.0313	0.1017	-0.0233	0.1102
PODL	**0.2762**	0.0820	0.1349	0.0157	0.1769
PDWP	**0.3221**	-0.0972	-0.0445	**-0.2116**	-0.0758
SDWPP	**0.3162**	-0.1598	-0.1174	-0.1540	-0.0283
SL	0.1891	-0.0179	-0.1687	**0.4418**	-0.0397
SW	0.1806	0.1260	**-0.2974**	**0.3198**	-0.0236
NSPP	**0.3264**	0.0117	**0.2577**	0.0407	0.0782
SDYLD	**0.3218**	-0.1188	-0.0761	-0.1053	0.0017
SCRP	0.0318	**0.2749**	0.0984	0.1419	0.1419
ECP	0.0885	**0.2100**	**-0.3350**	**-0.2126**	**0.2292**
TCV	-0.0805	-0.1072	**0.4274**	**0.2207**	-0.0879
BCVS	-0.0061	-0.1858	**0.3178**	0.0238	0.1588
TBPD	0.0124	**0.4405**	0.0378	-0.0821	-0.0877
TBSP	0.0124	**0.4405**	0.0378	-0.0821	-0.0877

N.B. Eigenvectors ≥ 0.2 are in bold.

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