

Genetic diversity analysis in medium duration pigeonpea [*Cajanuscajan* (L) Millsp.] germplasm for different agronomic traits and biotic factors

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Abstract

For the people living in tropical and sub-tropical regions, pigeonpea [*Cajanuscajan* (L) Millsp.] is a very essential pulse crop because of its high nutrition along with several important features such as feed and fodder for animal consumption, fuel for household work etc. Despite being a multipurpose and attractive crop, its productivity has remained up to 700-800kg ha⁻¹. Along-with low productivity, pigeonpea is also affected by a number of biotic stresses such as fusarium wilt, pod borer, pod fly. It is therefore, need of the hour to search for the genetic diversity present in the existing cultivars along with wild relatives and landraces. The present investigation was conducted with forty pigeonpea germplasm to assess the genetic diversity by principal component analysis (PCA). PCA is an important statistical technique which reduces the dimension of the much large data set into a more concise data set while retaining a significant amount of information from the original data. PCA analysis revealed a significant amount of variability present in the germplasm. PC1 contributed maximum variance towards diversity (22.05%) followed by PC2 (15.87%), PC3 (11.39%), PC4 (10.18%), PC5 (9.10%) and PC6 (8.18%). Scatter plot diagram showed that genotypes number 1 (CRG 82), 5 (GJP 1721), 19 (ICPL 15062), and 31 (BAUPP-18-8) exhibited the highest diversity.

Comment [WU2]: The benefits of human nutrition (food) should also be mentioned in the first.

Comment [WU3]: It is not necessary to explain the meaning of this method.

Key words: Genetic diversity, Principal Component Analysis, Pigeonpea.

Comment [WU4]: write the result of the diversity of biotic factors or the correlation between yield and biotic resistance capacity, because this is the main point of this study

Introduction

Pigeonpea [*Cajanuscajan* (L) Millsp.] is an important crop for small and marginal farmers living in tropical and sub-tropical regions across the world. It is an often-cross pollinated crop (30-70%) belongs to papilionaceae family. It is short lived perennial plant mostly grown as an annual crop. Pigeonpea plant is deep rooted with a height of 3 to 10 feet, slender leaves and exhibits large variation for flower and pod colour. It is a multi-purpose crop being used as human food (both green and mature peas), animal feeds such as seeds, leaves, husks and broken pods, also used as fuels in rural areas for household work. It is mostly used as split dals in most places. Being a deep-rooted crop, it helps to fix atmospheric nitrogen (30-50kg) (Krishna *et al.*, 2022) into the soil and sometimes it is also grown as a green manure crop because of its N-fixing ability. It serves as a vegetarian protein, containing almost 21-25% protein, for the poor people. Pigeonpea ranks sixth globally, however, in India it is the second important crop next to chickpea. India is the largest producer of pigeonpea and it alone accounts for more than 90% of total production in India followed by Myanmar and Malawi. It has an excellent ability to grow and yield well in less favourable conditions as well as drought prone depleted soils. Despite being such an attractive crop, its productivity remained low up to 700-800kg ha⁻¹ (Kumar *et al.*, 2017, Saxena *et al.*, 2020). The possible cause of this may be that the crop is affected by several biotic and abiotic stresses, lack of high yielding varieties, poor government policies, lack of proper knowledge about the cultivation practices, lack of good quality irrigation water etc. From the breeding aspect, first two causes are relevant and this could be overcome by searching

Comment [WU5]: This is a botanical property that should also be pointed out (regardless of the useful and other benefits)

Comment [WU6]: These sentences explain the general benefit and advantage, so you should add the references

Comment [WU7]: What is that? Are these units of nitrogen fixation per hectare? (30-50 kg N per ha?)

Comment [WU8]: You should move this sentence after the benefit as human food and add the references

Comment [WU9]: For pulse crops?

Comment [WU10]: Numerical statements are made and should be referenced. Other sentences in which numerical information is given contain the references.

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Comment [WU12]: except drought condition?

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for genetic diversity among the wild relatives and landraces as the present-day cultivars have narrow genetic base. Diversity analysis of released cultivars indicates limited amount of diversity being present in them. Genetic variability is the prerequisite to any crop improvement programme. Therefore, to increase the productivity of pigeonpea along with resistance/tolerance to multiple biotic and abiotic stress, it is essential study divergence among the available resources and select for the various economically important traits contributing to the divergence (Upadhyay *et al.*, 2007) in order to breed high yielding cultivars with resistance/tolerance to several biotic and abiotic stresses such as fusarium wilt, pod borer, pod fly, sterility mosaic disease which will result in sustainable nutritional security.

PCA is a method for reducing dimensionality which is commonly used to condense a large number of perplexing variables into a more concise set while retaining a significant amount of information from the original data (Hasan *et al.*, 2021). It is a statistical technique that employs an orthogonal transformation to change a collection of possibly interrelated variables into a set of linearly dissimilar variables called principal components. These principal components have a reduced number of data as compared to the original data set. The first principal component exhibits the maximum variation, while each subsequent component captures as much variation as possible, given that the orthogonal components are preceding components. The resultant vectors form an independent orthogonal basis set. The principal components exhibit orthogonality as they represent the eigenvectors of the symmetric covariance matrix. The main objective of the present investigation was to assess the genetic diversity using PCA among the pigeonpea germplasm.

Materials and methods

The present experiment was conducted during kharif 2021-22 and 2022-23 at the research farm of Birsa Agricultural University. The research site is located at an average altitude of approximately 625 meters (2,064 feet) above sea level. Geographically, it can be found at 23° 17' 50.4" N latitude and 85° 24' 39.4" E longitude in the Chhota Nagpur plateau region. The research farm is located within the tropical to sub-tropical climatic zone. The experiment consisted forty pigeonpea germplasm obtained from different sources (Table 1). The germplasm were grown in randomised block design (RBD) with two replications at a spacing of 60x30 cm. The plot size was 6.0m². Suggested agronomic practices were followed to raise a good crop stand along with biotic and abiotic stress management. Observations were recorded for days to 50% flowering, initial plant stand, final plant stand, plant height, days to maturity, 100 seed weight, number of pods/plant⁻¹, number of primary branches/plant⁻¹, number of secondary branches/plant⁻¹, wilt incidence%, pod borer incidence% from a sample of five randomly selected plants from each replication. However, yield (g plot⁻¹) and yield (kg ha⁻¹) were recorded on plot basis. The recorded data were pooled over two years and were subjected to further statistical analysis for PCA (principal component analysis). The software used for PCA was Indostat and biplot graph was prepared using R-studio software.

Comment [WU14]: write the full name (Principal Components Analysis; PCA)

Comment [WU15]: how to evaluate these traits

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Table 1. List of Forty medium duration pigeonpea germplasm

Comment [WU17]: What does this mean? planting age or other.

Sl. No.	Experimental materials	Source	Sl. No.	Experimental materials	Source
1.	CRG 82	TNAU, Coimbatore	21.	WRGE 309	ARS, Warangal
2.	GJP 1406	JAU, Junagarh	22.	WRGE 367	ARS, Warangal
3.	GJP 1502	JAU, Junagarh	23.	WRGE 369	ARS, Warangal
4.	GJP 1508	JAU, Junagarh	24.	BAUPP-20-49-1	BAU, Ranchi
5.	GJP 1721	JAU, Junagarh	25.	BAUPP-20-49-2	BAU, Ranchi
6.	GRG 82	JAU, Junagarh	26.	BAUPP-16-25-1-1	BAU, Ranchi
7.	KBA 32-3	IIPR Kanpur	27.	BAUPP-16-28	BAU, Ranchi
8.	IBTDRG-3	IIPR Kanpur	28.	BAUPP-16-30	BAU, Ranchi
9.	IBTDRG-2	IIPR Kanpur	29.	BAUPP-16-31	BAU, Ranchi
10.	IBTDRG-8	IIPR Kanpur	30.	BAUPP-16-33	BAU, Ranchi
11.	IBTDRG-10	IIPR Kanpur	31.	BAUPP-18-8	BAU, Ranchi
12.	IPA17-B-11	IIPR Kanpur	32.	BAUPP-17-44	BAU, Ranchi
13.	ICPL-151	ICRISAT, Hyderabad	33.	BAUPP-17-15-1	BAU, Ranchi
14.	ICPL-161	ICRISAT, Hyderabad	34.	BAUPP-20-33	BAU, Ranchi
15.	ICPL-7119	ICRISAT, Hyderabad	35.	BAUPP-20-35	BAU, Ranchi
16.	ICPL-15036	ICRISAT, Hyderabad	36.	BAUPP-20-46	BAU, Ranchi
17.	ICPL-15048	ICRISAT, Hyderabad	37.	BAUPP-20-31	BAU, Ranchi
18.	ICPL- 15048-1	ICRISAT, Hyderabad	38.	BAUPP-19-17	BAU, Ranchi
19.	ICPL-15062	ICRISAT, Hyderabad	39.	BAUPP-17-7	BAU, Ranchi
20.	Ormanjhi Local	BAU, Ranchi	40.	BAUPP-17-4-1	BAU, Ranchi

Results and discussions

The estimates on Principal component analysis for thirteen quantitative traits in pigeon pea (Table2)revealed that the recorded data from thirteen traits were transformed into six principal components. All the six principal components had eigen value more than one which showed 76.80% of the cumulative variation present in studied genotypes. 76.20% of cumulative variation was also found by Hamid *et al.*, (2011) for twelve traits. The eigen value of principal component first (PC1) (2.866) was highest, followed by PC2 (2.063), PC3 (1.481), PC4 (1.324), PC5 (1.184) and PC6 (1.063). The first principal component described the highest variation (20.053%) with high positive loadings for secondary branches (0.478) followed by plant height (0.407), yield (g/plot) (0.396), final plant stand (0.215), 100 seed weight (0.170), initial plant stand (0.051), number of pods plant⁻¹(0.047), and days to 50% flowering (0.040) whereas, wilt (%) (-0.217), primary branches (-0.016), days to maturity (-0.092), yield (kg/ha) (-0.552) were the negative contributors. Kumar *et al.*, (2021) also reported a variability of 21.45% explained by PC1 for plant height, grain yield per plant, number of pods plant⁻¹ in chickpea. Hemavathy *et al.*, (2017) found maximum variability by PC1 contributed by number of seeds pods⁻¹, plant height, 100 seed weight and number of secondary branches plant⁻¹

Comment [WU18]: 1. The numerical data presented should be verified.
2. There should be full references, such as plant species referred to in other studies.
3. The discussion should include more than just explaining how the results agree with other studies, e.g. what does it mean that some traits had positive and high values in each PC? What do negative values mean? And what does the finding of high scores for the same trait in many PC groups mean?

Comment [WU19]: What kind of plant?

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Comment [WU21]: Should you discuss this result, what are these characteristics?

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¹.PC2 revealed a total variation of 15.872%, which was positively contributed by number of pods plant⁻¹ (0.436), 100 seed weight (0.375), days to maturity (0.252), initial plant stand (0.237), plant height (0.173), days to 50% flowering (0.0358), final plant stand (0.0063). Rest of the traits had a negative loading. Hemavathy *et al.*, (2017), Hamid *et al.*, (2011) reported 16.09% of variability in PC2 contributed by number of seeds pods⁻¹ and 100 seed weight in pigeonpea.

Comment [WU24]: What kind of plant?

Comment [WU25]: how about pod borer (0.4363) and wit (0.420)?

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Comment [WU28]: What research are you referring to in this sentence?

Comment [WU29]: how about pod borer (0.3278)?

An additional 11.39% variation was revealed by PC3, which accounted positively for initial plant stand (0.393), final plant stand (0.187), primary branches (0.075), secondary branches (0.013), pod borer (%) (0.327), yield (kg/ha) (0.00007), but wilt (%) (-0.001), plant height (-0.067), days to 50% flowering (-0.705), number of pods plant⁻¹ (-0.142), days to maturity (-0.391), 100 seed weight (-0.094), and yield (g/plot) (-0.109) had negative loadings. Kumar *et al.*, (2021) also found negative loading for number of seeds pod⁻¹, number of primary branches plant⁻¹, number of secondary branches plant⁻¹, and days to maturity. Frimpomaah *et al.*, (2021) reported a variation of 9.20% for PC3. Nainu *et al.*, (2020) reported PC3 with negative loadings for 100 seed weight and number of pods cluster⁻¹. PC4 was mainly contributed by initial plant stand (0.461), final plant stand (0.033), plant height (0.075), secondary branches (0.161), days to 50% flowering (0.029), days to maturity (0.363), yield (g/plot) (0.148), and yield (kg/ha) (0.141) exhibiting positive factor loadings and negative loadings displayed by the rest of the traits in PC4. PC5 was explained by additional variance of 9.109% by highest positive loading for days to maturity (0.418) followed by initial plant stand (0.225), days to 50% flowering (0.221), primary branches (0.194), 100 seed weight (0.175), final plant stand (0.011), secondary branches (0.001), pod borer (%) (0.388). PC6 showed variability due to days to maturity (0.990), final plant stand (0.692), primary branches (0.413), number of pods plant⁻¹ (0.309), days to 50% flowering (0.210), yield (g/plot) (0.208), yield (kg/ha) (0.129), initial plant stand (0.056), with positive loadings, and plant height (-0.206), secondary branches (-0.048), and 100 seed weight (-0.257) revealed negative loadings.

Comment [WU30]: Discuss the meaning of positive or negative values in each PC

Comment [WU31]: what kind of plant?

Comment [WU32]: What is the discussion about the similarity or dissimilarity of the percentage in each PC in this study and others?

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Scatter plot and biplot PCA analysis

The PCA scores were calculated for forty pigeonpea genotypes across six principal components, which were then represented as three axes - X, Y, and Z. The squared distance of each genotype from these axes is given in Table 3. Subsequently, these three PCA scores for the forty pigeonpea genotypes were graphed to create a two-dimensional scatter diagram (Fig. 1). The results showed that genotypes number 1 (CRG 82), 5 (GJP 1721), 19 (ICPL 15062), and 31 (BAUPP-18-8) exhibited the highest diversity. The crossing of these diverse genotypes is expected to produce favourable transgressive segregants. Sudeepthi *et al.*, (2020) also reported two most diverse genotypes based on the scatter plot graph.

Comment [WU35]: 3 or 31, check again!

Comment [WU36]: diversity on the trait that interests you or not, especially a trait that is resistant or tolerant to biotic factors? please discuss.

Comment [WU37]: explain more, What kind of plant species? the number of varieties was tested and two or three or four genotypes showed diversity, what does that mean?

The biplot graph effectively represents relationship between the characters and genotypes, while also highlighting the significance of each character in contributing to the overall divergence. These relationships are depicted with the length of each vector which indicates the magnitude of contribution of a character on the genotype. The longer the vector, the greater the influence of the character and consequently more significant impact on the overall divergence between genotypes. On the other hand, characters with shorter vectors have a relatively lesser influence. This graph indicated that initial

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plant stand followed by final plant stand, yield (kg ha⁻¹) and number of pods plant⁻¹ contributed maximum to the total divergence [Fig 2]. Behera *et al.*, (2022), Alagupalamuthirsolai *et al.*, (2020) also validated their result through biplot analysis in rice.

The correlation between the traits is determined by the angle formed between the trait vectors. A 90° angle between the vectors signifies no correlation, while an acute angle (less than 90°) indicates a positive correlation, and an obtuse angle (greater than 90°) indicates a negative correlation. Plant height, final plant stand, days to 50% flowering, number of secondary branches plant⁻¹, number of primary branches plant⁻¹, number of pods plant⁻¹ and yield (g plot⁻¹) displayed a positive correlation with yield (kg ha⁻¹). Whereas, traits such as initial plant stand and 100 seed weight showed no correlation with yield (kg ha⁻¹). Days to maturity, pod borer incidence (%) and wilt incidence (%) showed negative correlation with yield (kg ha⁻¹) [Fig 2]. Similar positive correlations were observed by Kumar *et al.*, (2021) for days to 50% flowering.

The genotypes found within the trait vectors of the same quadrant typically demonstrate exceptional performance in terms of those specific characteristics. BAUPP-16-25-1-1, IBTDRG-3, WRGE 309, BAUPP-20-46 were found in the same quadrant performing best for plant height, days to 50% flowering, 100 seed weight, initial plant stand and final plant stand. IPA17-B-11 was found to be performing better for days to maturity. Ormanjhi local, IBTDRG-2, BAUPP-17-7, BAUPP-20-35 and KBA 32-3 was found to be highly affected by wilt incidence (%) and pod borer incidence (%). The genotypes which were found in more than 1 PCs indicated that the selection of these genotypes from these PCs would be helpful in further breeding programme. Similar findings were reported by Kumar *et al.*, (2021) and Akhande *et al.*, (2007)

Conclusion

The present investigation realised the presence of considerable amount of variability in the germplasm, which could be used in crop improvement programme. Principal component analysis revealed that traits i.e., initial plant stand plot-1, final plant stand plot, number of pods plant-1, 100 seed weight contributed majorly to yield present in the first three main PC. These traits contributed to 49.32% of total variability in the germplasm. PC1 contributed maximum variance towards diversity (22.05%) followed by PC2 (15.87%), PC3 (11.39%), PC4 (10.18%), PC5 (9.10%) and PC6 (8.18%). Scatter plot diagram showed that genotypes number 1 (CRG 82), 5 (GJP 1721), 19 (ICPL 15062), and 31 (BAUPP-18-8) exhibited the highest diversity. These genotypes could be used in further breeding programme. Biplot graph indicated that initial plant stand followed by final plant stand, yield (kg ha⁻¹) and number of pods plant⁻¹ contributed maximum to the total divergence and selection could be made for these traits for breeding for high yielding cultivars. Correlation studies revealed that plant height, final plant stand, days to 50% flowering, number of secondary branches plant⁻¹, number of primary branches plant⁻¹, number of pods plant⁻¹ and yield (g plot⁻¹) displayed a positive correlation with yield (kg ha⁻¹).

Table 2. Principal component analysis for thirteen quantitative traits in pigeon pea germplasm

SL.NO.	PC I	PC II	PC III	PC IV	PC V	PC VI
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Comment [WU39]: explain more for the results in this study

Comment [WU40]: add references

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Comment [WU44]: discuss these varieties because they show the diversity of biotic resistance factors

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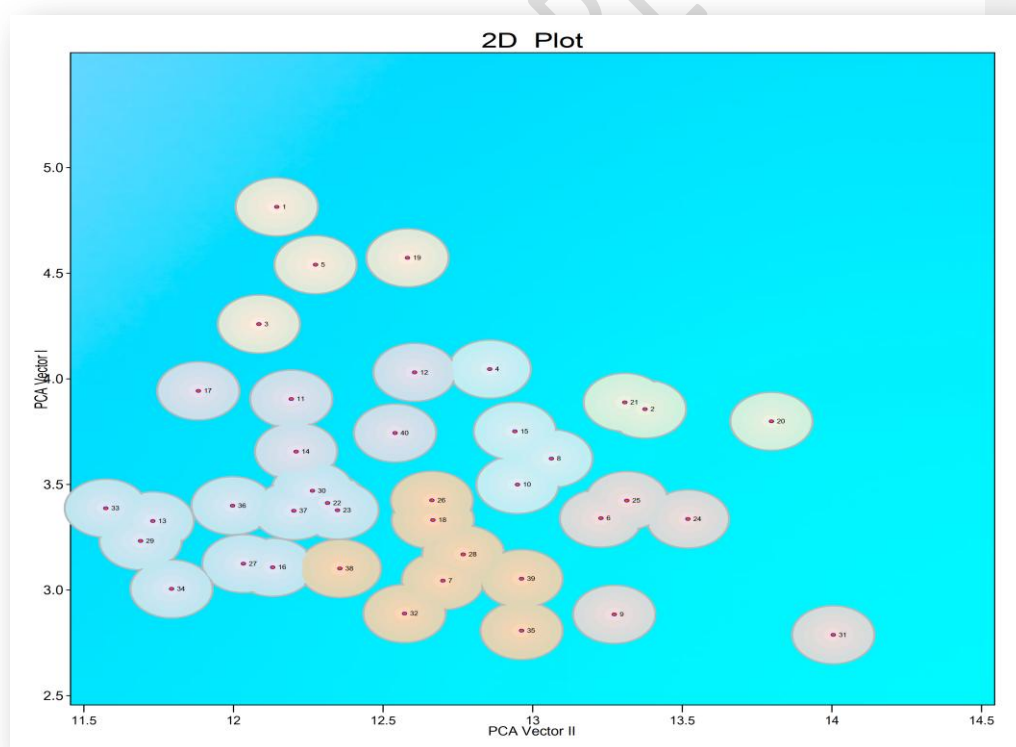
	Eigen Value (Root)	2.86692	2.06341	1.48171	1.32468	1.18430	1.06346
	% Var. Exp.	22.05322	15.87241	11.39776	10.18982	9.10996	8.18043
	Cum. Var. Exp.	22.05322	37.92563	49.32339	59.51321	68.62317	76.80360
1	Initial Plant Stand	0.05127	0.23733	0.39309	0.46190	0.22589	0.05685
2	Final Plant Stand	0.21531	0.00638	0.18707	0.03345	0.01198	0.69233
3	Wilt (%)	-0.21751	0.42006	-0.00194	-0.02256	-0.39999	0.11685
4	Plant height (cm)	0.40797	0.17329	-0.06774	0.07538	-0.29472	-0.20687
5	Primary Branches	-0.01602	-0.26686	0.07536	-0.52582	0.19411	0.41385
6	Secondary Branches	0.47899	-0.18742	0.01359	0.16114	0.00104	-0.04863
7	Days 50% flowering	0.04013	0.03581	-0.70559	0.02927	0.22182	0.21043
8	Number of pods per plant	0.04748	0.43630	-0.14243	-0.08438	-0.42836	0.30994
9	Pod Borer (%)	0.06117	0.44474	0.32778	-0.08777	0.38819	0.10312
10	Days to maturity	-0.09246	0.25228	-0.39175	0.36301	0.41880	0.09909
11	100 Seed weight (g)	0.17086	0.37588	-0.09455	-0.53604	0.17572	-0.25755
12	Yield (g)	0.39665	-0.15288	-0.10972	0.14897	-0.20533	0.20868
13	Yield (Kg/ha)	-0.55217	-0.11413	0.00007	0.14107	-0.18024	0.12917

Table 3. PCA scores for forty pigeonpea genotypes

SL.NO.	Genotype	PCA I	PCA II	PCA III
		X Vector	Y Vector	Z Vector
1	CRG 82	4.814	12.145	-14.427
2	GJP 1406	3.856	13.376	-13.041
3	GJP 1502	4.259	12.085	-13.574
4	GJP 1508	4.046	12.857	-13.963
5	GJP 1721	4.540	12.275	-14.942
6	GRG 82	3.340	13.228	-14.436
7	KBA 32-3	3.044	12.700	-14.881
8	BTDRG-3	3.622	13.063	-15.299
9	BTDRG-2	2.884	13.273	-14.768
10	IBTDRG-8	3.499	12.949	-14.915
11	IBTDRG-10	3.904	12.193	-14.998
12	IPA17-B-11	4.030	12.606	-14.605
13	ICPL-151	3.327	11.731	-14.186
14	ICPL-161	3.655	12.210	-13.560
15	ICPL-7119	3.751	12.941	-13.808
16	ICPL-15036	3.107	12.131	-14.926
17	ICPL-15048	3.943	11.883	-15.561
18	ICPL- 15048-1	3.331	12.667	-15.158
19	ICPL-15062	4.573	12.582	-15.029
20	Ormanjhi Local	3.799	13.798	-15.120
21	WRGE 309	3.888	13.308	-14.513
22	WRGE 367	3.412	12.315	-15.457
23	WRGE 369	3.378	12.348	-15.319
24	BAUPP-20-49-1	3.336	13.519	-14.486
25	BAUPP-20-49-2	3.423	13.315	-13.961
26	BAUPP-16-25-1-1	3.425	12.663	-14.573
27	BAUPP-16-28	3.124	12.034	-14.254

28	BAUPP-16-30	3.169	12.768	-14.020
29	BAUPP-16-31	3.232	11.690	-13.916
30	BAUPP-16-33	3.470	12.264	-14.230
31	BAUPP-18-8	2.788	14.005	-14.188
32	BAUPP-17-44	2.888	12.572	-14.100
33	BAUPP-17-15-1	3.387	11.573	-14.302
34	BAUPP-20-33	3.005	11.793	-15.003
35	BAUPP-20-35	2.808	12.963	-14.710
36	BAUPP-20-46	3.399	11.997	-13.922
37	BAUPP-20-31	3.375	12.202	-15.061
38	BAUPP-19-17	3.102	12.356	-13.724
39	BAUPP-17-7	3.053	12.963	-14.269
40	BAUPP-17-4-1	3.743	12.540	-13.959

Fig. 1. 2D graph of PCA scores of forty pigeonpea germplasms



Comment [WU48]: The number of varieties is too small, the size should be increased.

The image number and the name of the image should appear below the image, not above it.

References

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Comment [WU51]: Year?

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Comment [WU53]: Check the alphabetical ordering of references.

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Comment [WU54]: full name of Journal

Comment [WU55]: Journal abbreviation

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