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Principal Component Analysis and Clustering of Cassava Germplasm based on N and K Efficiency

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ABSTRACT

The present study was undertaken at ICAR-CTCRI, Sreekaryam to identify and group N and K efficient genotypes from a pool of released varieties, pre-breeding lines and elite landraces of cassava during 2021-2022. Thirty genotypes of cassava were evaluated for their diversity based on N and K efficiency along with some of its contributing plant characters using statistical tools like principal component analysis, and dendrogram clustering. The variation existing among the selected genotypes was observed through PCA, where the first six principal components accounted for nearly 81% of the total variability. Characters like tuber yield, plant height, stem girth, tuber length and tuber girth contributed to the greater variability among the genotypes. The dendrogram analysis classified the genotypes into six clusters based on the 18 parameters contributing to nutrient use efficiency. The proportion of the variance accounted by these clusters came up to the extent of 50% displaying the association of the genotypes with similar characters in these clusters. These analyses helped to realize the wide range of variability existing among the selected genotypes for the 18 characters studied. A simple correlation was also worked out between N and K use efficiency with root traits, which revealed that characters such as weight of storage roots, number of storage roots, and number of basal roots showed a positive correlation with both N and K use efficiency in cassava.

KEYWORDS: Cassava, dendrogram, N and K efficiency, PCA, pearson's correlation

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz), a prominent member of the family Euphorbiaceae, is one of the three major root and tuber crops cultivated globally and is the sixth largest source of energy in the worldIt has been aptly recognized as "food of the poor" by FAO (2013) and plays a vital role in maintaining global food and nutrition security, especially in developing and underdeveloped countries. More than 70% of its production is in tropical and subtropical regions, where it is largely grown as an annual crop for its edible starchy tuberous root, a rich source of carbohydrates. Although the crop is mainly used as human food, either fresh or in numerous processed forms (Lancaster et al., 1982, Jolayemi and Opabode, 2018, Fathima et al., 2022), its growing importance is largely attributed to its use as feed for animals and as a raw material for producing starch, starch-based products, and derivatives. Regionally, cassava is also a major source of bioethanol production (Nanbol and Namo, 2019).

Being a climate-resilient crop, cassava is normally cultivated in marginal soils with poor fertility giving reasonable yields, where many other crops do not grow well (Alves and Setter, 2004, Sandhu et al., 2021). Although it is grown in the tropics by poor farmers with minimum inputs, the crop has high nitrogen (N) and potassium (K) requirements. Long-term fertility trials suggest that sooner or later the deficiency of these vital nutrients would become the single most important factor limiting crop yield, especially in developing countries (Howeler et al., 2013). Among the primary nutrients, K plays the role of 'Key nutrient', as it helps in increasing tuber yield and enhances the tuber quality by improving its starch content. Moreover, it also assists in reducing the HCN content which causes bitterness in cassava tubers (John et al., 2014). Nitrogen is the second most important nutrient for cassava plants (Howeler, 2004, Omondi, 2019). It enhances the growth and development of plants in general, and also helps in improving photosynthetic efficiency and leaf area index, ultimately resulting in enhanced yield (Cock and Rosas, 1975, Howeler, 2004).

Cassava being highly responsive to manures and fertilizers is largely managed by resource-poor farmers through haphazard use of chemical fertilizers causing adverse environmental impacts (Pypers et al., 2012, Biratu et al., 2018). Hence, to reduce such economically wasteful and environmentally detrimental losses of fertilizer nutrients, farming systems now strive to boost the nutrient use efficiency (NUE) of cassava. NUE is the measure of the ability of a genotype to acquire nutrients from the growth medium and utilize it in the production of shoot and root biomass (Blair,1993). It is a combination of two major processes *viz.*, nutrient uptake efficiency and nutrient utilization efficiency (Hawkesford, 2011, Nitika et al., 2021). The efficient acquisition of nutrients depends largely on the root system architecture, rhizosphere nutrient mobilization and nutrient transporters (Baligar, 2001, Li et al., 2016, Lynch, 2019). Introducing nutrient efficient genotypes into a cropping system with low-input management strategies can reduce fertilizer use and thus agricultural costs. (Goulding et al., 2018). Identifying genotypes with maximum nutrient efficiency gives us a better understanding of the differences in nutrient uptake and response to fertilizer application and the dynamics of nutrients in the soil (Melvin et al., 2002). Owing to the importance of these nutrients in the overall growth and development of cassava, it is the need of the hour to explore the potential of N and K-efficient genotypes in reducing their respective fertilizer dosages. The current study mostly focuses on the identification of N and K-efficient genotypes from a pool of released varieties, pre-breeding lines and elite landraces of cassava.

2. MATERIALS AND METHODS

A total of 30 elite cassava genotypes including released and pre-release varieties from ICAR -Central Tuber Crops Research Institute and Kerala Agricultural University (Table 1) were used for the current study from September 2021- June 2022. They were planted at a spacing of 90×90 cm in the Block II of the experimental farm (latitude 8º 32"N; longitude 76º 65"E) at ICAR-CTCRI, in Randomized Block Design (RBD) with 3 replications with 10 plants each and without external application of fertilizers. The soil of the experimental site falls under the sandy clay loam texture with an acidic pH of 4.5–5.0, low in available N (below 240 kg ha^{-1}), high in available P (above 22 kg ha^{-1}), low in exchangeable K (below 110 kg ha^{-1}) and with medium organic matter (0.5 – 0.75). Plant characters such as plant height (cm), stem girth (cm), number of tubers $plant^{-1}$, tuber length (cm), tuber girth (cm), and tuber yield $plan^{-1}$ (t ha^{-1}), were recorded just before harvest of the crop. The height of the plant was measured from the base of the stem to the uppermost leaf tip, while the basal diameter of the longest branch was taken for the stem girth. Harvest was done nine months after planting and the above biometric observations were recorded from three randomly selected plants from each replication. The physiological efficiency (PE) of N and K was computed using the formula suggested by Soon (1992) to identify the N and K efficient genotypes.

PE (N or K)=Biological Yield (BY)/N or K update ……(1)

Biological yield (kg $plant^{-1}$) refers to the combined total of tuber and vegetative yields (*i.e* adding leaf, stem and tuber dry matter yields). It was computed on a dry weight basis for which 50 g of each plant part, leaves, stem and tubers

Table 1: List of genotypes and their sources

Gen-otypes	Name of Genotypes	Source
1	Sree Reksha	Released variety, ICAR-CTCRI
2	Sree Sakthi	Released variety, ICAR-CTCRI
3	Sree Pavitra	Released variety, ICAR-CTCRI
4	Sree Suvarana	Released variety, ICAR-CTCRI
5	Vellayani Hraswa	Released variety, KAU
6	Sree Vijaya	Released variety, ICAR-CTCRI
7	17S143	Breeding line, ICAR-CTCRI
8	Mulluvadi	Released variety, TNAU
9	17S247	Breeding line, ICAR-CTCRI
10	Sree Athulya	Released variety, ICAR-CTCRI
11	Ambakkadan	Landrace, Kerala
12	15S154	Breeding line, ICAR – CTCRI
13	KBH18	Exotic accession, ICAR -CTCRI
14	8S501-2	Pre-released variety, ICAR – CTCRI
15	7IIIE3-5	Breeding line, ICAR- CTCRI
16	CI-906	Landrace, ICAR-CTCRI
17	CI-905	Landrace, ICAR-CTCRI
18	15S351	Breeding line, ICAR-CTCRI
19	16-5	Breeding line, ICAR-CTCRI
20	8N113	Breeding line, ICAR-CTCRI
21	AVU13APink	Breeding line, ICAR-CTCRI
22	Export Kappa	Landrace, Tamil Nadu
23	Karutha Malabar	Landrace, Kerala
24	Kumkumarose	Landrace, Kerala
25	17S39	Breeding line, ICAR-CTCRI
26	15S409	Breeding line, ICAR-CTCRI
27	17S120	Breeding line, ICAR-CTCRI
28	11S17	Breeding line, ICAR-CTCRI
29	17S36	Breeding line, ICAR-CTCRI
30	17S48	Breeding line, ICAR-CTCRI

were oven dried at 65±5°C till stable results were obtained. The dry matter percentage was obtained from the dry and fresh weight of each plant part. For computing N and K uptake, their respective content in leaf, stem and tuber were determined following Kjeldahl's method (Veibel, 1949) and flame photometry (Singh et al., 2005). For deducing each plant part's N or K uptake, the dry matter yield was multiplied by their respective N or K content. Total N or K uptake was calculated by summing the leaf, stem and tuber N or K uptake (Anonymous, 1984). Tuber starch content (%) was determined following the anthrone method (Hansen and Møller, 1975) and was expressed on a fresh weight basis. Statistical analysis like Principal Component Analysis (PCA), Biplot, cluster and dendrogram analysis were performed using SAS 9.3 software. A simple correlation study (Pearson's correlation) was also done to understand the relation of N and K use efficiency with the root traits viz. number of nodal roots, number of basal roots, number of storage roots, number of adventitious roots, length of roots, weight of storage roots, weight of basal and nodal roots.

3. RESULTS AND DISCUSSION

3.1. Principal component analysis (PCA)

Principal component analysis (PCA) reflects the essentiality of the major contributor towards the total variation at each of its axis of differentiation (Sharma, 1998). It is a linear combination involving the simplification of complex data.

PCA of the present investigation revealed that out of the eighteen characters studied, only six principal components exhibited an Eigenvalue greater than 1, which accounts for an aggregate percentage of 80.99% variation for the selected genotypes. Therefore, it can be concluded that all the variations in the data can be explained by the first six principal components. The contribution of eighteen characters to the six principal components are presented in table 2.

The variation percentage between Eigenvalues and the principal components is represented in the scree plot (Figure 1). In this study, PC1 showed 26.33 % variability with an eigenvalue of 4.739 followed by PC2 (20.53%) with an

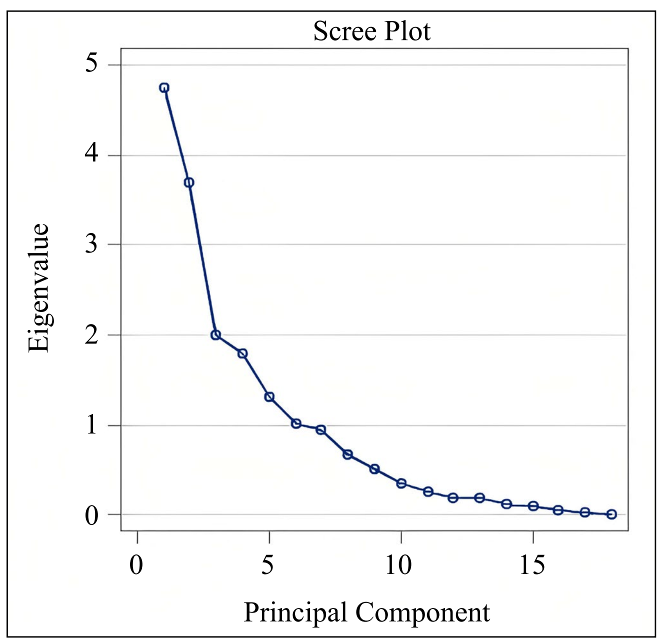

Figure 1: Screeplot diagram

Table 2: PCA analysis of traits taken for the study in cassava genotypes

Variable	PC1	PC2	PC3	PC4	PC5	PC6
Eigen value (Root)	4.739	3.695	2.004	1.797	1.322	1.021
Percentage Variation expressed	26.330	20.530	11.130	9.980	7.340	5.670
Cumulative variation expressed (%)	26.330	46.860	57.990	67.980	75.320	80.990
Plant height	0.230	0.269	0.230	0.121	0.056	0.494
Stem girth	0.208	0.345	0.173	-0.216	0.177	0.192
Tuber yield	0.333	0.266	-0.051	0.100	0.046	-0.201
Number of tubers	0.227	0.288	-0.177	0.166	0.134	-0.180
Tuber length	0.202	0.269	0.014	0.143	-0.356	0.336
Tuber girth	0.213	0.106	0.026	-0.059	0.580	-0.390
Tuber starch content	-0.282	-0.107	-0.041	0.151	0.484	0.171
Leaf dry matter content	0.040	0.102	0.368	0.494	-0.087	-0.143
Stem dry matter content	-0.057	-0.091	0.297	0.566	0.061	-0.129
Tuber dry matter content	-0.325	-0.084	0.070	0.232	0.314	0.374
Leaf N Percentage	-0.326	0.297	0.119	-0.117	-0.088	-0.119
Stem N Percentage	-0.222	0.205	0.321	-0.214	0.089	0.092
Tuber N Percentage	-0.300	0.278	0.169	-0.059	-0.051	-0.256
Leaf K Percentage	-0.164	-0.164	0.197	-0.067	-0.278	-0.143
Stem K Percentage	0.216	-0.194	0.322	-0.323	0.197	0.142
Tuber K Percentage	0.233	-0.226	0.331	0.049	-0.065	-0.166
N efficiency	0.248	-0.346	-0.238	0.155	0.016	0.132
K efficiency	-0.176	0.301	-0.445	0.194	0.019	0.034

eigenvalue of 3.695 and PC3 (11.13 %), PC4 (09.98%), PC5 (7.34%), PC6 (5.67%) with eigenvalues of 2.004, 1.797, 1.322 and 1.021 respectively. It is clear from the scree plot diagram that the maximum contribution of variation was due to PC1 when compared to other PCs. For identifying characters that have a major influence on the PCA value, it is relevant to look for individual loadings. The values that are closer to the unit value in a considered component will represent the influential characters in that particular principal component (Bhanupriya et al., 2014). Figure 2, depicts the 2D plot of the principal components (PC1 and PC2) which exhibits the major contribution towards total variation among the selected genotypes. Genotypes that are plotted in an extreme positive direction in both the axis (X and Y) represent the superior ones and as the distance between the genotypes increases, it reflects the decrease in similarity among the genotypes.

The characters which showed significant contribution in the PC1 are tuber yield (0.333), plant height (0.230), N efficiency (0.248), tuber K percent (0.233) and the characters like tuber starch content, plant dry matter content (stem+leaf+tuber), K efficiency etc exhibited negative

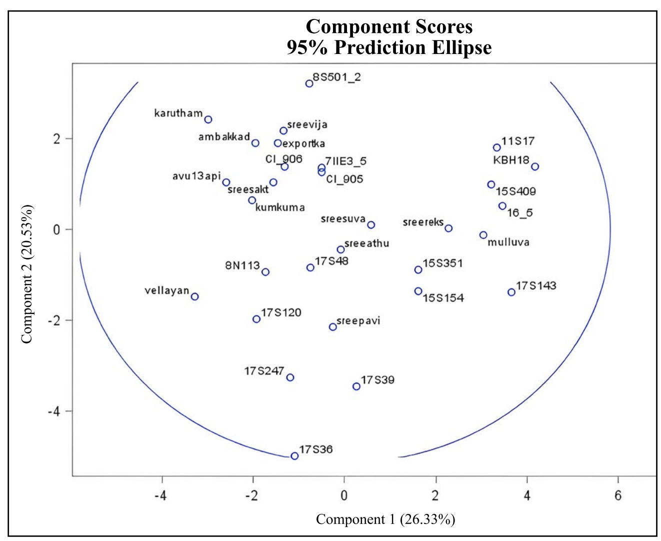

Figure 2: 2D plot distribution of 30 genotypes based on the principal component PC1 (X axis) and PC2 (Y axis)

loadings. John et al. (2020) reported similar findings with regard to tuber yield and plant height in PC1. In PC2, stem girth (0.345), K efficiency (0.301), and leaf N % (0.297) had a greater contribution while the characters' tuber starch content, dry matter content, etc. again showed negative loadings. The principal component PC3 seemed to be largely influenced by the characters, leaf dry matter content (0.368), stem N percentage (0.321%) and tuber K percentage (0.331%). In PC4 also, leaf and stem dry matter content (0.494, 0.566) depicted a significant contribution. The principal component 5 was majorly contributed by the tuber girth (0.580), tuber starch content (0.484), and tuber dry matter content (0.314) while the characters, leaf and tuber N percentage and leaf N and tuber K percentage demonstrated negative loadings. The characters, plant height (0.494), tuber length (0.336) and tuber dry matter content (0.374) noted an effective contribution to PC6.

The 3D plot distribution (Figure 3) displays the diversity among the selected genotypes based on the nutrient use efficiency using the three principal components (PC1, PC2 and PC3). The variability existing among the genotypes can be observed based on the different colours chosen to

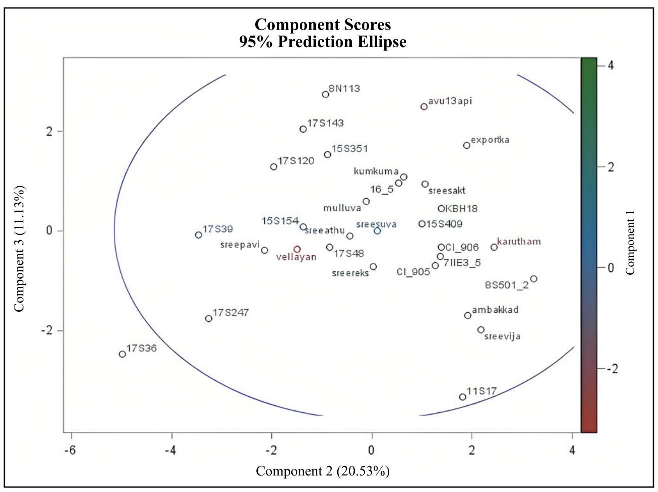

Figure 3: 3D plot distribution of 30 genotypes based on the principal component PC1 (Y axis), PC2 (X-axis), and PC3 (Z axis)

represent the genotypes in the 3D plot.

3.2. *Dendrogram clustering*

Using dendrogram analysis, the 30 cassava genotypes were classified into different clusters based on 18 parameters contributing to nutrient use efficiency. Based on this, the genotypes were grouped into two main principal clusters. The two main clusters consisted of 6 sub-clusters (Figure 4). The first principal cluster consisted of subclusters I and II with 13 genotypes *viz.* Ambakkadan, 7IIIE3-5, Sree Vijaya, Karutha Malabar, AVU13A Pink, 8N113, CI-905, Export Kappa, CI-906, Kumkumrose, Vellayani Hraswa, 8S501-2 and Sree Sakthi. The second cluster comprised subclusters III-VI and the 17 genotypes included in these clusters are, 17S36, 17S39, Sree Suvarna, KBH18, 17S48,

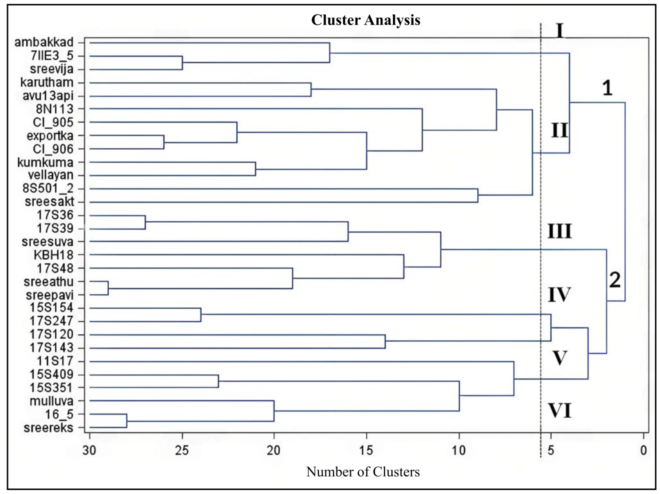

Figure 4: Cluster dendrogram depicting the genetic relationship between 30 cassava genotypes

Sree Athulya, Sree Pavitra, 15S154, 17S247, 17S120, 17S143, 11S17, 15S409, 15S351, Mulluvadi, 16-5 and Sree Raksha. When the data were grouped into six clusters, the proportion of variance accounted for by the clusters came up to just under 50%.

3.3. *Correlation studies*

A Pearson's correlation was also worked out between the N, and K efficiency with the root traits, to understand the effect of root characters on nutrient use efficiency in cassava. The weight of storage roots has a maximum positive correlation with nitrogen use efficiency, followed by the number of storage roots, number of adventitious roots, weight of basal & nodal roots, number of nodal roots, root length and number of basal roots (Table 3). So, it is evident that these root characters have a direct association with the nitrogen use efficiency in cassava. Liu et al. (2022) reported that an increase in the number of embryonic roots contributed to nitrogen use efficiency in wheat. On the other hand, potassium use efficiency showed a positive correlation with the weight of storage roots, number of storage roots,

Table 3: Pearson's correlation coefficients between N & K use efficiency with root traits

	N efficiency	K efficiency
No. of nodal roots	0.133	-0.207**
No. of storage roots	0.241**	0.195**
No. of basal roots	0.114	0.051
No. of adventitious roots	0.188**	-0.206**
Length of roots	0.116	-0.134
Weight of storage roots	0.321**	0.203**
Weight of basal & nodal roots	0.134	-0.320**

**: Significant at (p=0.01) level of probability and number of basal roots and a negative correlation with number of nodal roots, root

length, number of adventitious roots and weight of basal & nodal roots. Similar conclusions were made by John et al. (2020) who revealed that the number of tuberous roots indeed has a positive effect on potassium use efficiency in cassava. Further studies on root traits could pave the way for breeding improved nutrient-use-efficient traits.

4. CONCLUSION

The presence of broad genetic variability along with good genotypic characteristics is an important criterion for the development of efficient varieties through any crop improvement programme. In this study, an attempt was done to evaluate and classify genotypes based on their various plant characters as well as their N and K use efficiency and this was carried out with the help of different statistical tools like PCA and dendrogram clustering. N and K efficient genotypes includes 17S36, 17S39, Ambakkadan, 7IIIE3-5, Sree Vijaya, Karutha Malabar etc.

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