Egyptian Journal Of

GENETICS AND CYTOLOGY

INTERNATIONAL JOURNAL DEVOTED TO GENETICAL AND CYTOLOGICAL SCIENCES

Published by

THE EGYPTIAN SOCIETY OF GENETICS

Volume 53 **January 2024** **No. 1**

BREEDING FOR ENHANCED YIELD AND QUALITY TRAITS IN COWPEA (*Vigna unguiculata* L.)

T. A. EL-AKKAD[1,2*], ENTSAR M. E. ABO-HAMDA[3], AND AMANI H. A. M. GHARIB[3]

[1]Department of Genetics and Genetic Engineering, Faculty of Agriculture, Benha University, Benha, Egypt.

[2]Moshtohor Research Park, Molecular Biology Lab, Benha University, Benha, Egypt.

[3]Horticulture Research Institute, Agriculture Research Center, Giza, Egypt.

*Corresponding author: tamer.abdelghaffar@fagr.bu.edu.eg

Key words: *Cowpea,Vigna unguiculata, P.C.V, G.C.V, Heritability, IRAP, Inter Retrotransposon Amplified Polymorphism.*

Cowpea (*Vigna unguiculata* L. Walp.; 2n =2x = 22) is a pivotal crop cultivated extensively in low-in put production systems and arid and semi-arid agro-ecologies globally (Boukar *et al*., 2019). As a legume within the family Fabacea and sub-family Faboideae (Padulosi and Ng, 1997 and Agbogidi, 2010), cowpea, characterized by low outcrossing and high self-pollination, serves as a valuable source of low-cost protein (17 to 25%) with essential amino acids, lysine and tryptophan (Rangel *et al.*, 2003; Ibro *et al.*, 2014). Recognized as the "poor man's

meat" in many developing countries (Jayathilake *et al.,* 2018), cowpea thrives in challenging environmental conditions, contributing to soil fertility through nitrogen fixation in crop rotation (Bado *et al.,* 2006; Dugje *et al.,* 2009 and Gnanamurthy *et al*., 2012).

Driven by rapid population growth in Egypt, recent research focuses on enhancing cowpea yield quantity and quality through intensive breeding efforts, reliant on the presence of genetic variability enabling effective selection. The selection of superior genotypes correlates with the extent of genetic variability and the heritability of the inherited characteristics (Scarano *et al*., 2014). Understanding the magnitude and type of genetic variability, along with corresponding heritability, is crucial in breeding programs for improving crop yield and quality traits. Therefore, investigating the relationship between genotype variability and yield components is essential for the efficient utilization of cowpea genetic resources in the context of Egyptian agricultural productivity.

In most crop improvement programs, enhancing yield stands as a primary breeding objective (More and Borkar, 2016). Cowpea yield, being a quantitative trait, is intricately linked to numerous morphological, physiological and agronomic traits, influenced by both genetic and environmental factors. The efficacy of selection relies on the availability of substantial genetic variability within the breeding material for the target character and its heritability (Atta *et al.,* 2008). The direction and magnitude of associations between traits to be improved also play a crucial role (More and Borkar, 2016). Thus, studying the genetic variability and heritability of yield and its associated traits is paramount for yield improvement. Plant genetic resources exhibit variation that supports the selection of superior genotypes and the development of improved cultivars with desirable characteristics.

Protein markers and DNA markers can be used for assessment genetic variability based on morphological traits which influenced by environmental factors (El-Shazly *et al.,* 2020). Retrotransposons are ubiquitous and abundant transposable elements in eukaryotic genomes which classified into long terminal repeats (LTRs) and non-LTRs (Kumar and Bennetzen, 1999). Retrotransposons are dispersed throughout plant genomes and some retrotransposon families are represented by thousands of copies (Kalendar *et al.,* 2010). New copies of retrotransposons are randomly inserted into preexisting sequences of the genome *via* a copy-paste system, which consequently increases the copy number (Kalendar and Schulman, 2007). Retrotransposons contribute to the size, structure, variation, and diversity of the genome. In addition, they greatly effect gene function and cover a high percentage of the genome (Gbadegesin and Beeching, 2010).

They are known to insert themselves into the genome and act as muta-

genic agents thereby providing a potential source of gene diversity (Bourque *et al.*, 2018). Among the transposable element based markers, new retrotransposon-based DNA fingerprinting techniques, IRAP (Inter Retrotransposon Amplified Polymorphism) that produce dominant, multiplex marker systems that examine variation in retrotransposon insertion sites. IRAP makes use of conserved retrotransposon sequences termed LTRs for detection of polymorphism. It is based on the amplification of regions between two neighboring retrotransposon.

IRAPs serve as effective molecular markers owing to the abundance of retrotransposon copies in plant genomes and their ability to generate new copies (Kalendar and Schulman, 2013). RTN markers possess advantages of easy assessment, low cost, and high in formativeness and polymorphism (Bhandari *et al*., 2017). Consequently, IRAP markers provide an efficient DNA fingerprint for each genotype, enabling genetic identification and kinship assessment (Badr, 2008). The effectiveness of IRAP analysis has been demonstrated in various studies, such as those on *Medicago sativa* L. landraces and Iranian bread wheat cultivars and breeding lines (Annicchiarico, 2006; Nasri *et al*., 2013; Farouji *et al*., 2015 and Taheri *et al*., 2018). Additionally, IRAP has been applied in phylogenetic analyses among commercial triploids and their wild relatives in Musa germplasm (Somasundaram *et al*., 2023) and for fingerprinting, diversity studies, and linkage maps in yeast and barley (Shehata *et al*., 2015).

Despite extensive literature, there is a dearth of reports on the use of IRAP markers to assess the genetic diversity of cowpea (*Vigna unguiculata* L.) genotypes in Egypt. This study aims to fill this gap by providing insights into the genetic diversity of the country's cowpea germplasm using IRAP markers. Additionally, the research evaluates genetic variability and heritability among cowpea genotypes for yield and related traits, aiming to identify promising lines with maximum productivity and high seed quality under Egyptian conditions.

MATERIALS AND METHODS

This research was conducted over the span of 2022 to 2023 under open field conditions at Qaha Vegetable Research Farm, Horticultural Research Institute (HRI), Agriculture Research Center (ARC), situated in Qalyoubia Governorate, Egypt. A comprehensive selection of 20 breeder-chosen lines of cowpea (*Vigna unguiculata* L. Walp) and five commercially established cultivars (Balady, Cream 7, Kafr Elsheikh 1, Qaha 1, and Tiba) were utilized in this study. All entries were sourced from the Horticultural Research Institute, Agriculture Research Center (ARC), Egypt. The identification of promising lines was based on criteria such as earliness, seed quality, and high seed yield, as illustrated in Table (1) and Fig. (1). It is noteworthy that there was observed variation in seed color among these entries.

The evaluation took place over two consecutive summer seasons in 2022 and

2023, with combined data across both seasons being calculated. The seeds of the twenty-five genotypes (comprising twenty selected lines and five commercial cultivars) were sown in the first week of May during both seasons. A randomized complete block design with three replicates was employed, with each plot consisting of three rows. The seeds were sown on raised beds, maintaining an 80 cm row-to-row spacing and a 15 cm plant-to-plant spacing at a depth of 5 cm. Standard cultural practices, including irrigation, chemical fertilization, and disease and pest control, were applied in accordance with local practices. Data were systematically collected and recorded on a plot basis, with the mean of each genotype utilized in subsequent statistical analyses. The parameters studied encompassed the number of days to flowering, pod length (cm), number of seeds per pod, 100-seed weight (g), and seed yield (ton/feddan; where one feddan equals 4200 m^2).

Genomic DNA extraction, purification and quantification of 25th cowpea genotypes

Due to the high protein content in cowpea varieties, high molecular weight genomic DNA was isolated from fresh leaves of 25th cowpea genotypes using DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) with some minor modifications by adding PVP (poly venial pyrolidine) to help eliminate phenols, dyes and part of proteins. The quantity and purity of extracted DNA were assessed spectrophotometrically using the ND-1000 system (Nano-Drop Technologies, Thermo Fisher Scientific Inc., Waltham, MA, USA) according to the Molecular Cloning Laboratory Manual (Maniatis *et al.*, 1988).

IRAP primers - PCR analysis

The IRAP assay, following the methodology outlined by Badr *et al*. (2020), was employed to assess genetic variation within and among 25 cowpea genotypes, utilizing a set of 10 primers (refer to Table 2). The IRAP PCR amplification reactions were conducted in uniform 20µl volumes, comprising 10µl of 2xMaster Mix (One PCRTM, GeneDireX, Inc., Taipei, Taiwan), 2µl of DNA template (15 ng/µl), 2.5µl of primer (10 pc/mol/µl), and 5.5µl of dH_2O.

Amplification reactions were carried out using a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems), with the programmed conditions detailed in Table (3). The resulting amplification products were separated through electrophoresis in a 3% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 120 volts. Visualization of PCR products was achieved under UV light, and images were captured using a Gel Documentation System (BIO-RAD 2000).

Statistical analysis

The acquired data underwent statistical analysis within each season, and subsequently, a combined analysis was performed after confirming the homogeneity of seasons using the method outlined by

Gomez and Gomez (1984). Mean comparisons were conducted using Duncan's multiple range test (Duncan, 1955). The coefficient of variance was computed following the procedure outlined by Steel and Torrie (1981). Genotypic and phenotypic coefficients of variation were estimated based on Burton's methodology (1952). Broad-sense heritability was determined in accordance with the approach proposed by Singh and Chaudhary (1995). The heritability percentage was categorized into low (0-30%), moderate (30–60%), and high (≥60%), following the classification by Johnson *et al.* (1955).

RESULTS AND DISCUSSION

Performance of the selected lines

Table (4) presents a comprehensive overview of the performance of the studied cowpea genotypes for the traits number of days to flowering, pod length, and number of seeds per pod during the 2022 and 2023 seasons, as well as a combined analysis across both seasons. The results reveal substantial variation among the genotypes for each trait.

For the number of days to flowering, considerable diversity was observed, with recorded values ranging from 48.33 days (Line CP 23-1) to 58.83 days (Line CP 66 and cultivar Kafr Elsheikh 1). The overall mean of the selected lines was 53.01 days, while the check cultivars exhibited an overall mean of 55.03 days. This disparity suggests the effectiveness of the selection process in improving the trait.

In the case of pod length, significant differences were noted among the genotypes. CP 25-3 displayed the longest pods (18.04 cm), followed by Kafr Elsheikh 1 and Cream 7 cvs. (17.63 and 17.52 cm, respectively). Notably, CP 23 and CP 25-2 exhibited the shortest pods (12.94 cm and 13.87 cm, respectively), emphasizing the distinctiveness among the studied genotypes.

The number of seeds per pod also exhibited significant differences among the genotypes. Kafr Elsheikh 1 demonstrated the highest number of seeds per pod (13.47), followed by the line CP 25-3 (12.47) without a significant difference between them. These findings emphasize the effectiveness of the selection process in enhancing the seeds per pod trait.

Moving on to the 100-seed weight trait (Table 5), significant differences were evident among the genotypes, with the mean weight ranging from 11.83 g to 18.01 g. The line CP 67 showcased the heaviest seeds (18.01 g), while the line CP 57 exhibited the lowest value (11.83 g). These variations underscore the diverse seed weights among the cowpea genotypes.

The final trait, seed yield per feddan, also displayed significant differences among the genotypes. The selected line CP 65 demonstrated the highest seed yield per feddan (1.456 ton), followed by Tiba (1.431 ton). This suggests the efficacy of the selection process in enhancing seed

yield, with substantial differences observed among the check cultivars.

These results align with previous studies by Ahmed *et al.* (2005), Hussein and Abd El-Hady (2015), and Adams *et al.* (2017), which identified significant differences among cowpea genotypes for traits such as days to 50% flowering, pod length, number of seeds per pod, 100-seed weight, and seed yield. Additionally, Gomes *et al.* (2021) emphasized the high morphological diversity in local landraces, while Boukar *et al.* (2019) attributed the narrow genetic diversity in cowpea to its self-pollinating nature and limited gene flow between wild and cultivated types. Similarly, Lopes *et al.* (2003) and Dalorima *et al.* (2014) emphasized the potential for trait improvement through selection in cowpea.

Components of variances

Table (6) provides comprehensive estimates of various components of variance for the studied traits, including environmental (σ^2_e), genetic (σ^2_g), and phenotypic (σ^2_p) variance, as well as genotypic (GCV) and phenotypic (PCA) coefficients of variation, GCV/PCV ratios, and broad-sense heritability (BSH).

With the exception of the number of seeds per pod, all studied traits exhibited minimal differences between phenotypic and genetic variance (Table 6). This indicates that a substantial portion of the phenotypic variance (σ^2_p) can be attributed to genetic variance (σ^2_g), emphasizing the genetic nature of the significant differences observed among the cowpea selected lines.

Analysis of the data in Table (6) reveals low discrepancies between phenotypic and genotypic variance for most studied traits, as evidenced by high GCV/PCV ratios ranging from 0.66 to 0.89. This suggests that a major proportion of the phenotypic variance (σ^2_p) is underpinned by genetic factors (σ^2_g). Furthermore, the estimated broad-sense heritability exhibited moderate to high values (ranging from 43.64% to 79.28%) across all traits, underscoring that the observed significant phenotypic differences among the studied breeding lines predominantly result from genetic factors, with minimal environmental effects on phenotypic variation, except for the trait number of seeds per pod. Consequently, the investigated traits are poised for improvement through selection based on phenotypic observations in early segregating generations. These findings align with the research of Ahmed *et al.* (2005), who documented elevated GCV and PCV for traits such as number of seeds per pod, seed yield, 100-seed weight, and pod length. Additionally, they noted high heritability for seed yield and 100-seed weight, indicating a prevalence of additive gene effects for these traits, however, the heritability for the number of days to 50% flowering was estimated at 31.83%. Similarly, Damarany (1994) and Gomes *et al.* (2021) reported high heritability values, suggesting that early-generation selection can effectively be applied for traits such as seed weight and 100-seed weight.

Assessment of polymorphism in 25th cowpea genotypes using IRAP markers

The evaluation of polymorphism within and among 25th selected cowpea genotypes, comprising 5 commercial cultivars and 20 newly developed lines, was conducted employing ten IRAP primers. The chosen primers demonstrated high efficiency, successfully amplifying bands and providing substantial information.

The amplification reactions resulted in multiple band profiles, generating 7 to 15 amplified DNA fragments per primer, with an average of 11 bands. Notably, the number of polymorphic fragments ranged from 2 to 9, averaging 1.1 polymorphic bands per primer. Primer IRAP-4375 exhibited the highest polymorphic fragments (9), while IRAP-2198 and IRAP-4351 displayed the minimum (2), as detailed in Table (7).

In total, the ten primers produced 108 reproducible fragments, of which 44 were polymorphic, indicating a considerable polymorphism level of 40.7% among the studied cowpea genotypes. The size of the amplified fragments varied between 100 and 1800 bp, as visualized in Figs. (2 & 3).

Moreover, the analysis identified unique markers capable of distinguishing between cowpea genotypes. Among the ten primers, IRAP-4352, IRAP-2198, and IRAP-2200 did not generate unique markers. In contrast, the remaining seven primers produced distinctive markers, including both unique positive and/or negative markers for cowpea genotype identification.

Notably, four IRAP primers (IRAP-2204, IRAP-4340, IRAP-4370, and IRAP-4375) generated both unique positive and negative markers, while three IRAP primers (IRAP-2197, IRAP-4351, and IRAP-3471) produced only unique positive markers. In total, twelve unique markers were identified from the ten IRAP primers, comprising eight unique positive and four unique negative markers, with molecular weights ranging from 100 to 1600 bp, as summarized in Table (7). These findings highlight the robustness of the IRAP marker system in discerning genetic variations among the cowpea genotypes studied.

Assessment of genetic relationships in 25th cowpea genotypes using IRAP markers

Understanding genetic relationships is paramount in the management of primary gene pool collections for efficient germplasm utilization in breeding and conservation programs, especially in the face of environmental changes. Molecular markers, being unaffected by environmental factors, offer a reliable estimate of genetic diversity, a crucial prerequisite for effective breeding initiatives. The calculation of genetic distances and subsequent dendrogram construction using the UPGMA method is a common practice in fingerprinting to organize germplasm efficiently and enhance genotype sampling.

In our study, we utilized IRAP marker data for 25 cowpea genotypes, creating a genetic distance tree based on Dice's genetic similarity matrix (Fig. 4). The tree revealed distinct clustering patterns, with Balady cv. forming a solitary branch, and the remaining genotypes segregating into two main clusters. Further analysis through Principal Component Analysis (PCA) scatter plots illustrated the differentiation of genotypes, highlighting unique positions for Balady and Kafr Elshaikh 1 cvs., while Qaha 1 cv. exhibited discernible distances from most other genotypes (Fig. 5).

Multivariate heatmap analysis, using the R software, reinforced the clustering observed in the genetic distance tree. Two major clusters emerged, each comprising specific genotypes. Notably, Balady cv. formed a cluster with CP 35-1, CP 23-1, CP 23, and CP 35, while Qaha 1 cv. clustered with CP 56-1, CP 65, CP 25-2, CP 56, and CP 25-3 (Fig. 6).

The findings from the IRAP analysis underscore the existing genetic differences among key cowpea varieties traded in Egyptian markets. This diversity enabled the development of promising new varieties, as evidenced by the high genetic similarity among certain new lines and Qaha 1 cv., a parent used in hybridization. Notably, CP 56-1, CP 65, CP 25-2, CP 56, and CP 25-3 demonstrated superiority in various morphological and productive traits compared to local Qaha 1 and Balady cvs., confirming their grouping in the same genetic category.

Our study aligns with the work of Sarr *et al*. (2020) and Xiong *et al*. (2016), demonstrating significant genetic variation within and among cowpea genotypes. Additionally, Dagnon *et al*. (2022) emphasized the importance of genetic diversity assessment for effective conservation programs. These results further validate the robustness of IRAP markers in discerning genetic relationships, as seen in other studies involving diverse plant species like *Medicago sativa* (Mandoulakani *et al*., 2012); Asian bamboo (Shitian *et al*., 2020); *Hordeum vulgare* (Kalendar and Schulman, 2014); *Citrus* (Abedinpour *et al*., 2014); *Lallemanti aiberica* (Cheraghi *et al*., 2018) and *O. europaea* (Khaleghi *et al*., 2017).

CONCLUSION

Based on the comprehensive analysis of the collected data, it is evident that the promising lines, namely CP 25-2, CP 25-3, CP 56, CP 56-1, and CP 65, exhibit noteworthy characteristics that make them strong contenders for certification pending further evaluations. These identified lines demonstrate not only high productivity but also exhibit favorable yield components and early maturity, coupled with the desirable seed color. The robustness of this recommendation is substantiated by genetic testing, which elucidated the specific genetic position occupied by these promising varieties within the broader spectrum of cowpea cultivars traded in Egypt. The analysis further highlighted the internal variations existing among these genotypes, underscoring their significance as

valuable sources for the development of distinct and innovative cultivars. This intrinsic diversity becomes especially crucial in addressing environmental challenges and bridging nutritional gaps, reinforcing the potential of these cultivars to contribute significantly to sustainable agricultural practices and food security.

SUMMARY

This investigation was carried out at Qaha Vegetable Research Farm, ARC, Qalyoubia Governorate, Egypt spanning from 2022 to 2023,with the aim of exploring the genetic variability and heritability of key economic characters while developing promising cowpea *(Vigna unguiculata* L.) lines. The study incorporated twenty novel lines alongside five commercially established cowpea cultivars. Notably, the results underscored that a substantial proportion of the phenotypic variance (σ^2_p) was attributable to genetic variance (σ^2_g), excluding the trait related to the number of seeds per pod. Moreover, the broad-sense heritability estimates demonstrated moderate to high values (ranging from 43.64% to 79.28%) across all scrutinized traits. This suggests that the discernible phenotypic variations among the genotypes were predominantly of genetic origin, with minimal environmental impact on the observed phenotypic diversity. Consequently, the potential for enhancing these traits through selection based on early segregating generations is highlighted. Genetic diversity of cowpea genotypes estimated using IRAP markers (Inter Retrotransposon Amplified Polymorphism). The total number of reproducible fragments amplified by the ten primers reached 108 bands, of which 44 were polymorphic fragments. This represented a level of polymorphism of 40.7%, which indicates a very high level of polymorphism among the studied cowpea genotypes. Noteworthy lines, such as CP 25-2, CP 25-3, CP 56, CP 56-1, and CP 65, exhibited promising attributes, including high productivity, favorable yield components, earliness, and desirable seed color. These lines are earmarked for potential certification pending further evaluations, showcasing their potential contribution to enhanced cowpea cultivation.

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Table (1): Assessed cowpea genotypes throughout the2022 and 2023 seasons, along with their distinctive characteristics.

#	Genotype	Source	Growth habit	Flower colour	Seed color	Distinctive characters
1	**CP 23**	Qaha 1 X Balady	Erect	Purple	Creamy with brown eye	Small-sized seeds
2	**CP23-1**	Qaha 1 X Balady	Erect	Purple	Brown	Early maturity
3	**CP 25**	Qaha 1 X Balady	Erect	White	White with brown eye	Early maturity, small-sized seeds
4	**CP 25-2**	Qaha 1 X Balady	Erect	White	Creamy with brown eye	High yielding, early maturity, small-sized seeds
5	**CP 25-3**	Qaha 1 X Balady	Erect	Purple	brown	High yielding, early maturity, large-sized seeds
6	**CP 30-1**	Qaha 1 X Balady	Erect	White	Creamy with brown eye	Large-sized seeds
7	**CP 35**	Qaha 1 X Balady	Semi-erect	Purple	Brown	High number of seeds/pod
8	**CP 35-1**	Qaha 1 X Balady	Semi-erect	Pink	Creamy with brown eye	Early maturity
9	**CP 52**	Qaha 1 X Tiba	Erect	White	White with brown eye	Good seed color
10	**CP 52-1**	Qaha 1 X Dokki 331	Erect	White	brown	Good seed color
11	**CP 56**	Qaha 1 X Dokki 331	Erect	White	Creamy with brown eye	High yielding
12	**CP 56-1**	Qaha 1 X Dokki 331	Trailing	Purple	Creamy with brown eye	High yielding, early maturity, large-sized seeds
13	**CP 57**	Qaha 1 X Dokki 331	Erect	Dark purple	Black	Early maturity, small-sized seeds
14	**CP 64**	Qaha 1 X Dokki 331	Erect	White	White with Black eye	Early maturity
15	**CP 65**	Qaha 1 X Dokki 331	Semi-erect	White	Creamy with brown eye	High yielding, early maturity, large-sized seeds
16	**CP 65-1**	Qaha 1 X Dokki 331	Semi-erect	Purple	Brown	Early maturity, large-sized seeds
17	**CP 66**	Qaha 1 X Dokki 331	Erect	White	White with Black eye	High yielding, large-sized seeds
18	**CP 67**	Segregation from cv. Qaha 1	Erect	White	White with Black eye	Early maturity, large-sized seeds
19	**CP 67-1**	Segregation from cv. Qaha 1	Erect	White	Brown	Small-sized seeds
20	**CP 70**	Segregation from cv. Tiba	Erect	White	Brown	Large-sized seeds
21	**Balady**	Landrace	Trailing	Dark purple	brown	High yielding, small-sized seeds,
22	**Cream 7**	HRI[z]	Erect	White	Creamy	Late maturity
23	**Kafr El-shaikh 1**	HRI	Erect	White	Creamy	Late maturity, large-sized seeds
24	**Qaha 1**	HRI	Erect	White	Creamy with brown eye	Early maturity, small-sized seeds
25	**Tiba**	HRI	Erect	White	Creamy with brown eye	High yielding, early maturity

HRI[z]: Horticultural Research Institute

Table (2): The sequence information for the 10 primers used in the IRAP-PCR marker assay.

#	Primers	Sequence 5’ to 3’
1	IRAP4352	ACCCGGAAGGGCGGTTCATGCAA
2	IRAP-2198	ATCCTTCGCGTAGATCAAGCGCCA
3	IRAP 2197	GAAGTACCGATTTACTTCCGTGTA
4	IRAP 2200	ATGTGACAGTCGACTAACCAC
5	IRAP 2204	TACCCTTTTAAGGGATCAACC
6	IRAP 4351	AACTTGATCCAGATCATCTCC
7	IRAP 4340	ATGGTTGTCGAAACTCCAGC
8	IRAP 4370	ATGCCGTATTCTCAGCATCC
9	IRAP 4375	ATCGCTCCGGGTGCCTAACAC
10	IRAP 3471	ATCGCTCCGGGTGCCTAACAC

Table (3): IRAP -PCR reaction parameters.

	Temperature	Time period	Cycle
Initial denaturation	94ºC	5min	1
Denaturation	94ºC	50 Sec	
Annealing	54ºC	55 sec	35
Extension	72ºC	1.3 min	
Final extension	72ºC	10 min	1

Table (4): Mean performance of genotypes for number of days to flowering, pod length andnumber of seeds per pod characters.

Genotypes	Characters								
	Number of days to flowering			Pod length (cm)			Number of seeds/pod		
	2022	2023	Combined	2022	2023	Combined	2022	2023	Combined
CP 23	55.67 a-d	53.67 d-f	54.67 b-d	13.29 jk	12.54 g	12.94 I	10.70 b	10.53 bc	10.62 de
CP23-1	49.00 g	47.67 i	48.33 j	14.82 f-j	15.33 c-f	15.08 d-h	12.07 ab	12.53 a	12.30 ab
CP 25	52.00 d-g	50.67 gh	51.33 g-i	12.50 kl	17.09 ab	14.80 e-i	12.00 ab	12.50 ab	12.25 a-c
CP 25-2	52.67 d-g	50.67 gh	51.67 f-i	11.73 l	16.02 a-e	13.87 hi	11.33 ab	11.97 a-c	11.65 b-e
CP 25-3	51.00 e-g	49.67 hi	50.33 i	19.16 a	16.91 a-c	18.04 a	12.67 ab	12.27 a-c	12.47 ab
CP 30-1	54.00 b-f	52.67 e-g	53.33 c-f	16.49 b-e	16.09 a-e	16.29 a-e	10.83 b	10.50 c	10.67 c-e
CP 35	54.00 b-f	52.67 e-g	53.33 c-f	15.61 d-h	16.19 a-e	15.90 b-h	11.43 ab	12.00 a-c	11.72 b-e
CP 35-1	51.67 d-g	50.67 gh	51.17 g-i	15.10 e-i	16.06 a-e	15.58 c-h	11.53 ab	11.73 a-c	11.63 b-e
CP 52	55.00 a-e	53.67 d-f	54.33 b-e	16.87 b-d	16.03 a-e	16.45 a-e	12.13 ab	11.10 a-c	11.62 b-e
CP 52-1	57.67 ab	57.67 ab	57.67 a	16.88 b-d	15.01 d-f	15.95 b-g	11.33 ab	10.80 a-c	11.07 b-e
CP 56	54.00 b-f	52.67 e-g	53.33 c-f	14.30 h-j	16.51 a-d	15.41 d-h	11.40 ab	11.43 a-c	11.42 b-e
CP 56-1	51.67 d-g	50.67 gh	51.17 g-i	14.60 g-j	14.99 d-f	14.80 e-i	11.23 b	10.77 a-c	11.00 b-e
CP 57	53.00 c-g	50.67 gh	51.83 f-i	13.98 i-k	14.33 f	14.16 f-i	10.43 b	10.53 bc	10.48 e
CP 64	52.33 d-g	52.67 e-g	52.50 e-h	15.37 d-i	15.33 c-f	15.35 d-h	10.47 b	10.50 c	10.48 e
CP 65	52.00 d-g	50.67 gh	51.33 g-i	15.90 d-g	15.19 d-f	15.55 c-h	11.80 ab	12.07 a-c	11.93 a-e
CP 65-1	51.33 e-g	50.67 gh	51.00 g-i	15.93 d-g	15.17 d-f	15.55 c-h	11.50 ab	12.07 a-c	11.78 b-e
CP 66	59.00 a	58.67 a	58.83 a	16.18 c-f	15.66 b-f	15.92 b-g	11.77 ab	11.47 a-c	11.62 b-e
CP 67	50.67 fg	51.00 f-h	50.83 hi	16.56 b-e	17.49 a	17.03 a-d	12.20 ab	12.27 a-c	12.23 a-d
CP 67-1	55.00 a-e	55.67 b-d	55.33 b	15.84 d-g	16.30 a-e	16.07 a-f	12.33 ab	12.33 a-c	12.33 ab
CP 70	57.00 a-c	58.67 a	57.83 a	16.33 c-f	17.00 a-c	16.65 a-e	11.87 ab	12.53 a	12.20 a-d
Balady	55.67 a-d	54.67 c-e	55.17 bc	13.37 jk	14.67 ef	14.02 g-i	10.70 b	11.07 a-c	10.88 b-e
Cream 7	58.33 a	56.67 a-c	57.50 a	17.98 ab	17.05 ab	17.52 a-c	11.60 ab	11.80 a-c	11.70 b-e
Kafr Elshaikh 1	59.00 a	58.67 a	58.83 a	17.57 bc	17.69 a	17.63 ab	14.20 a	12.73 a	13.47 a
Qaha 1	51.00 e-g	50.67 gh	50.83 hi	16.43 c-e	16.48 a-d	16.46 a-e	11.07 b	11.50 a-c	11.28 b-e
Tiba	53.00 c-g	52.67 e-g	52.83 d-g	16.10 c-g	16.13 a-e	16.12 a-f	12.07 ab	12.43 a-c	12.25 a-c

Means with the same letter are not significantly different from each other

Table (5): Mean performances of selected cowpea lines and check cultivars for 100 seed weight and seed yield/fed. characters.

Genotypes	Characters					
	100- seed weight			Seed yield/fed. (ton/fed.)		
	2022	2023	Combined	2022	2023	Combined
CP 23	13.97 e-g	14.03 f-j	14.00 f-j	0.464 i	0.403 h	0.434 j
CP23-1	15.45 c-e	14.16 f-j	14.81 d-i	0.756 e-g	0.780 c-g	0.768 d-f
CP 25	12.85 gh	14.60 d-i	13.72 f-j	0.755 fg	0.735 c-h	0.745 e-g
CP 25-2	13.37 f-h	12.82 h-k	13.09 g-j	1.032 bc	1.086 bc	1.059 b
CP 25-3	18.31 ab	15.11 c-g	16.71 a-e	1.002 bc	0.983 cd	0.993 bc
CP 30-1	18.79 a	15.52 c-g	17.15 a-d	0.598 g-i	0.593 e-h	0.596 f-j
CP 35	15.54 c-e	13.79 f-j	14.66 e-i	0.752 fg	0.765 c-h	0.758 d-f
CP 35-1	14.70 d-f	13.51 g-k	14.11 f-j	0.561 hi	0.584 e-h	0.573 g-j
CP 52	15.61 c-e	14.36 e-j	14.98 c-g	0.467 i	0.451 gh	0.459 j
CP 52-1	16.20 cd	14.99 c-h	15.59 b-f	0.542 hi	0.525 f-h	0.533 ij
CP 56	15.42 c-e	18.47 a	16.94 a-e	0.933 b-e	0.920 c-e	0.927 b-d
CP 56-1	18.93 a	15.71 c-g	17.32 a-c	0.941 b-d	0.989 cd	0.965 bc
CP 57	12.18 h	11.47 k	11.83 j	0.544 hi	0.583 e-h	0.564 h-j
CP 64	15.22 c-e	14.50 d-j	14.86 d-h	0.491 i	0.473 gh	0.482 j
CP 65	18.76 a	15.25 c-g	17.01 a-e	1.449 a	1.464 a	1.456 a
CP 65-1	18.37 a	15.00 c-h	16.68 a-e	0.469 i	0.477 gh	0.473 j
CP 66	18.09 ab	17.20 a-c	17.65 ab	0.966 bc	0.987 cd	0.976 bc
CP 67	19.26 a	16.76 a-d	18.01 a	0.871 c-f	0.864 c-f	0.868 c-e
CP 67-1	11.84 h	12.55 i-k	12.20 j	0.766 d-g	0.768 c-h	0.767 d-f
CP 70	16.67 bc	16.49 a-e	16.58 a-e	0.537 hi	0.550 e-h	0.543 ij
Balady	12.53 gh	12.30 jk	12.42 ij	1.088 b	1.073 bc	1.080 b
Cream 7	15.43 c-e	12.37 i-k	13.90 f-j	0.671 gh	0.674 d-h	0.672 f-i
Kafr Elshaikh 1	16.33 cd	18.07 ab	17.20 a-d	0.539 hi	0.571 e-h	0.555 ij
Qaha 1	12.30 gh	12.83 h-k	12.56 h-j	0.747 fg	0.722 c-h	0.735 e-h
Tiba	15.83 cd	15.87 b-f	15.85 a-f	1.428 a	1.433 ab	1.431 a

Means with the same letter are not significantly different from each other

Table (6): Variance components (σ^2_p,σ^2_gandσ^2_e), genotypic (GCV) and phenotypic (PCA) coefficient of variation and broad sense heritability (BSH%) for cowpea traits.

Variance Compo-nents	Characters				
	No. days to flowering	**Pod length**	**No. seeds/ pod**	**100 seeds weight**	**Seed yield /fed.**
ó^2_e	2.303	0.509	0.500	0.923	0.025
ó^2_g	8.816	1.094	0.387	2.999	0.073
ó^2_p	11.119	1.603	0.887	3.922	0.098
H^2_{Bs} (%)	79. 28	68.25	43. 64	76. 46	74. 87
G.C.V. (%)	0.0036	0.0163	0.0187	0.0294	1.6937
P.C.V. (%)	0.0041	0.0198	0.0284	0.0336	1.9574
G.C.V/ P.C.V	0.89	0.83	0.66	0.87	0.87

Table (7): Levels of polymorphism, total number of bands, monomorphic bands, polymorphic bands, percentage of polymorphism, unique positive and unique negative bands as revealed by IRAP markers among the 25th cowpea genotypes.

No.	Primers	Total number of bands	Mono morphic bands	Poly morphic bands	% p	UPM	MW bp	UNM	MW bp
1	**IRAP4352**	7	4	3	43	0		0	
2	**IRAP-2198**	9	7	2	22	0		0	
3	**IRAP 2197**	12	8	4	33	2(L5)	600, 700	0	
4	**IRAP 2200**	13	9	4	31	0		0	
5	**IRAP 2204**	9	5	4	44	1(L14)	150	1(CV25)	180
6	**IRAP 4351**	14	12	2	14	1(L8)	150	0	
7	**IRAP 4340**	11	4	7	64	1(L4)	1600	1(CV23)	1400
8	**IRAP 4370**	8	3	5	63	1(L4)	900	1(1)	180
9	**IRAP 4375**	15	6	9	60	1(L9)	150	1(CV25)	220
10	**IRAP 3471**	10	6	4	40	1(L8)	1600	0	
Total		**108**	**64**	**44**	**407**	**8**		**4**	
Average		**10.8**	**6.4**	**4.4**	**40.7**	**0.8**		**0.4**	

Fig. (1): Illustrates the compilation of cowpea genotypes examined during the 2022 and 2023 seasons. Genomic DNA extraction, purification and quantification of 25th cowpea genotypes.

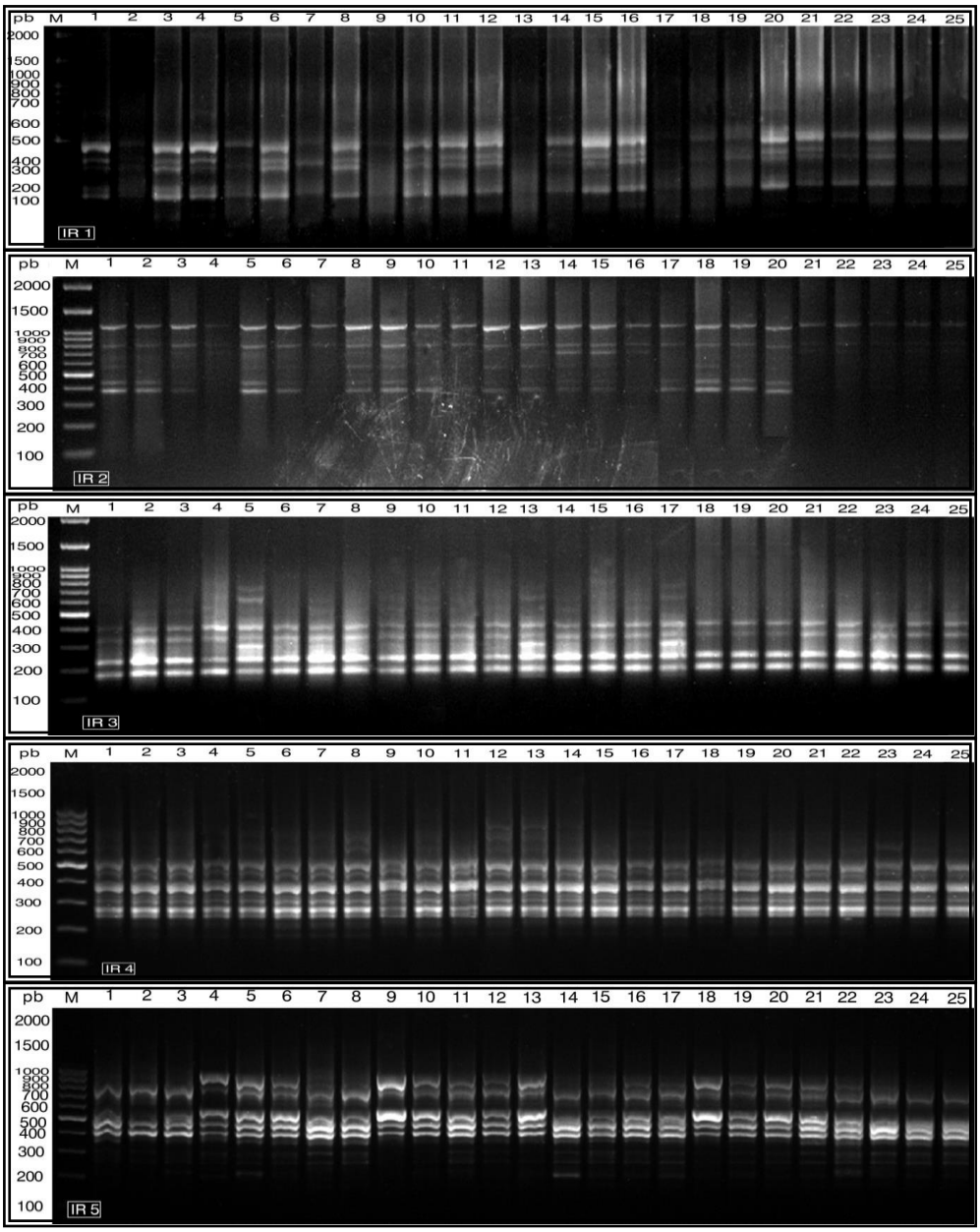

Fig. (2): IRAP profiles of 25th cowpea genotypes (1 - 25) as detected with primers (1) IRAP4352, (2) IRAP-2198, (3) IRAP 2197, (4) IRAP 2200 and (5) IRAP 2204. DNA molecular weight standards (M) 100 bp DNA ladder.

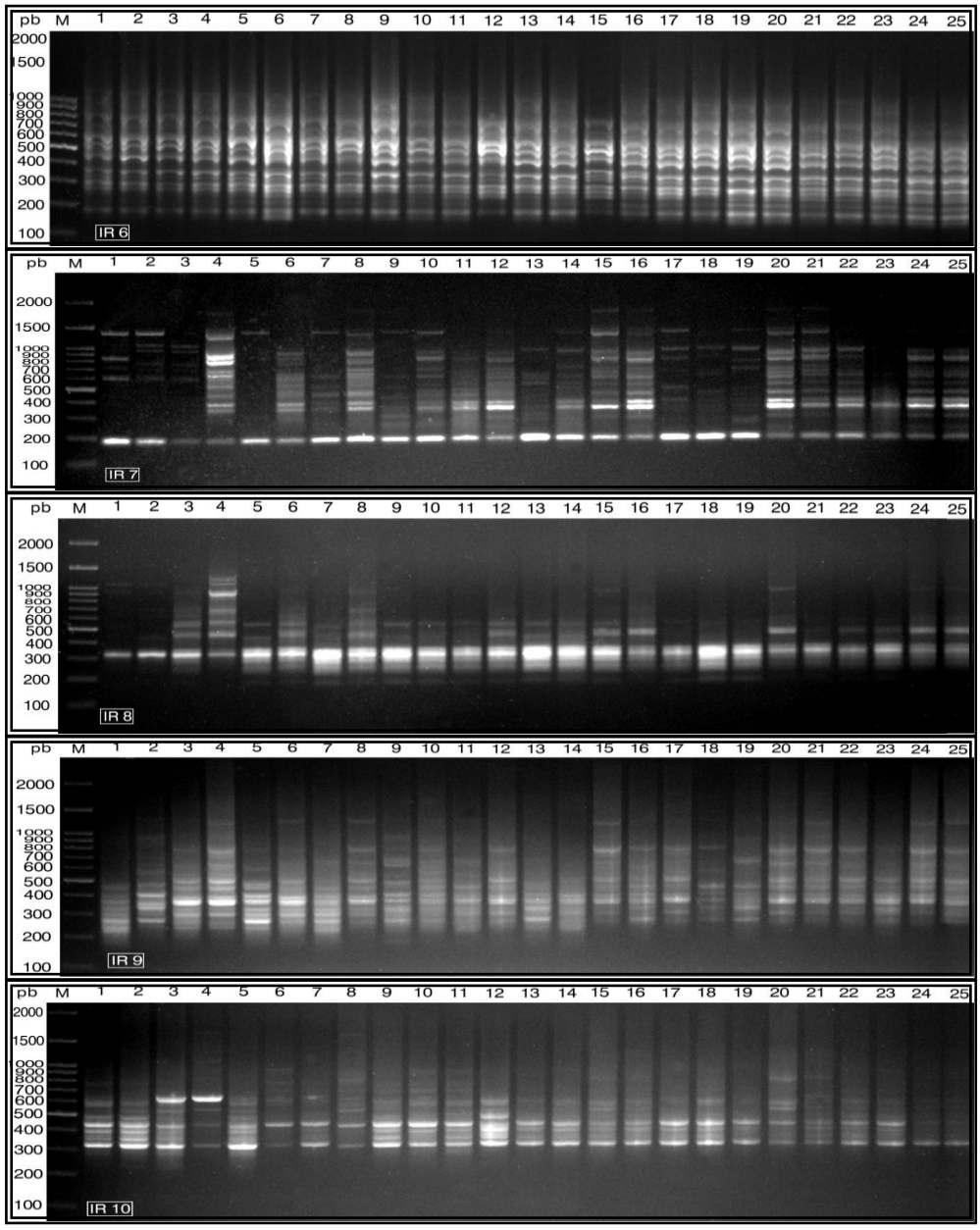

Fig. (3): IRAP profiles of 25th cowpea genotypes (1 - 25) as detected with primers (6) IRAP 4351, (7) IRAP 4340, (8) IRAP 4370, (9) IRAP 4375 and (10) IRAP 3471. DNA molecular weight standards (M) 100 bp DNA ladder.

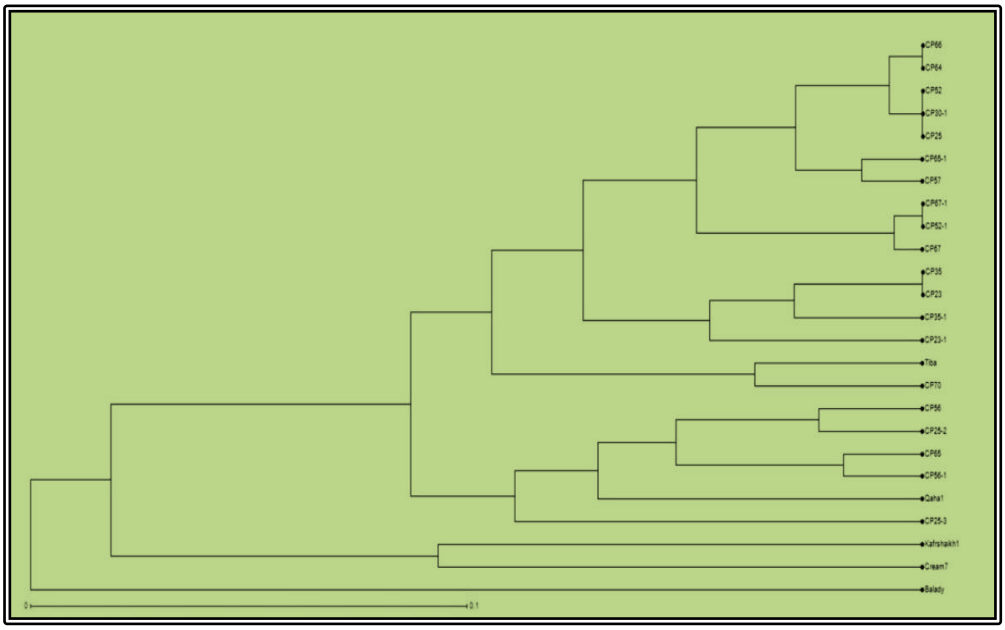

Fig. (4): Cluster tree illustrating the relationship of 25th cowpea genotypes based on the analysis ofIRAP marker polymorphism, constructed using the Euclidean similarity matrices computed as Dicecoe_cients and using the unweighted pair group method with arithmetic mean (UPGMA) algorithmin the PAST software.

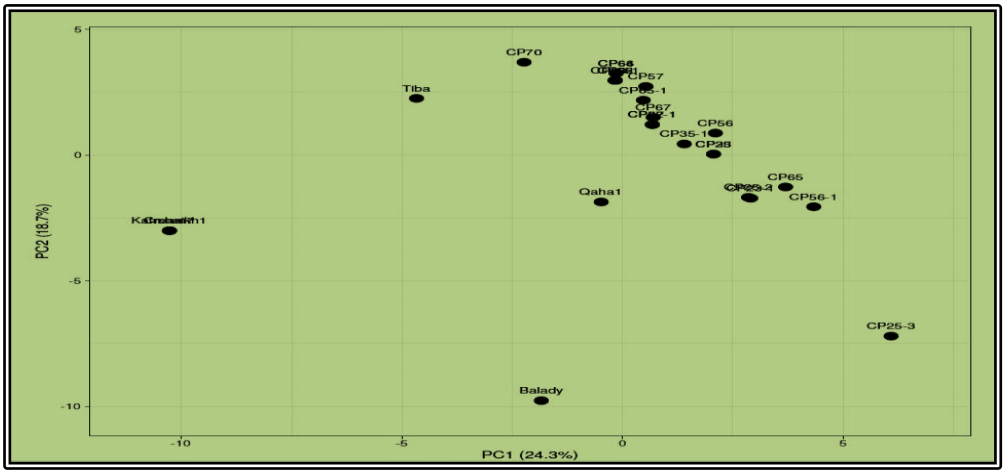

Fig. (5): The PCA analysis (principle component analysis) scatter diagram illustrating the genetic diversityexpressed by the grouping of of 25th cowpea genotypes based on the analysis of IRAP marker polymorphism and by blotting the first two principale components using PAST software.

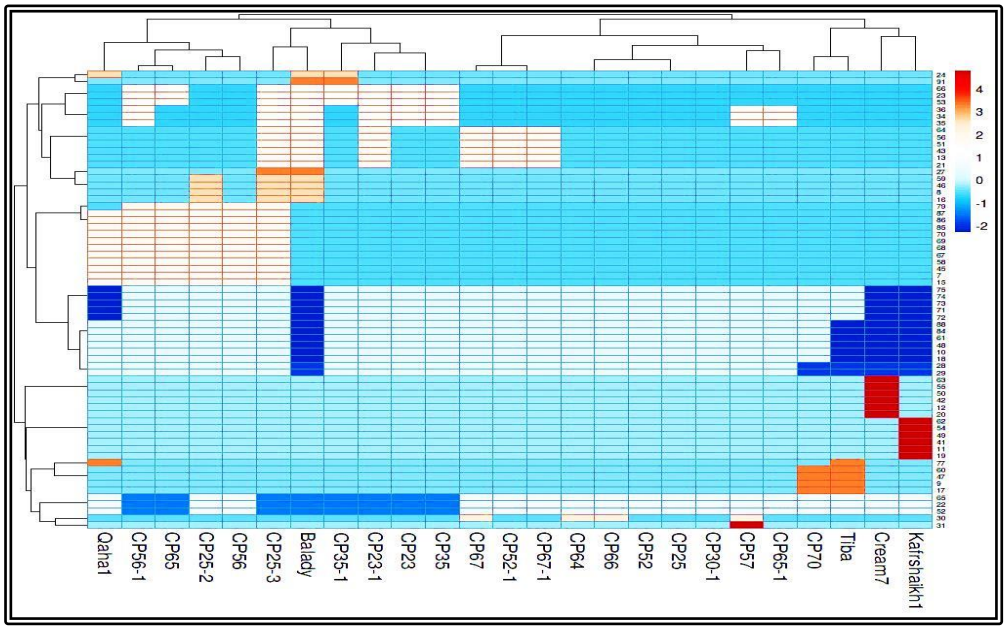

Fig. (6): Multivariate heatmap illustrating the genetic diversity of of 25th cowpea genotypes based on the IRAP markers constructed using the module of heatmap of R software.