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STUDY ON QUALITATIVE, QUALITY AND DNA FINGER PRINTING PROFILE OF IDENTIFIED BLACK PEPPER GERMPLASM UNDER LOWER BRAHMAPUTRA VALLEY ZONE OF ASSAM CONDITIONS OF NORTH EAST REGION, INDIA

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ABSTRACT

Spices adds flavor and aroma to many households around the world. The North East region (NER) of India boasts an excellent fertile soil and conducive environment for various spices including black pepper, particularly in the state of Assam, where black pepper holds a pivotal position as a lucrative cash crop. The importance of local genotypes and landraces in increasing the genetic diversity of modern crop varieties, and thus enhancing their economic value, is well recognized. In the present study, thirteen local black pepper germplasm were collected and evaluated along with check variety Panniyur 1 for its qualitative, quantitative and quality traits. The germplasm IC-0599150 was identified as the highest yielder with better quality traits. The DNA finger printing with 50 ISSR markers was carried out to distinguish the germplasm IC-0599150 with Panniyur 1. ISSR-01 marker distinguished the Panniyur 1 and IC-0599150 with the presence of band at ISSR-011000 and absence of band at ISSR-01510. Hence, the accession IC-0599150 was identified as suitable accession for NEH region, particularly for Assam state and it was proposed for the variety release.

Keywords: Black pepper, germplasm, qualitative traits, quality, DNA finger printing

Introduction

Black pepper botanically known as *Piper nigrum* L., holds a revered status in the culinary world, celebrated for its distinctive flavor and aroma, originating from the tropical regions of South East Asia, particularly India. Indian pepper fetches a premium price in major international markets due to its preference and intrinsic qualities (Thomas, 2010). In India, it is grown in an area of 1,38,929 ha with a production of 48,000 t (Spices Board, 2020). Kerala and Karnataka are the largest producers of black pepper; recently the North East region of India emerges as a notable contributor to the cultivation and production of this esteemed spice (Krishnamoorthy and Parthasarathy, 2009).

Historically, black pepper has held significant cultural and economic importance in the North East region. Its trade routes connected the region to distant lands, facilitating commerce and cultural exchange. The cultivation of black pepper emerged as a crucial source of livelihood for local communities, contributing to the region's socio-economic development (Tripathy *et al*., 2018).

In recent years, there has been a growing emphasis on organic and sustainable farming practices in the North East region. Farmers in the NER are embracing eco-friendly cultivation practices for black pepper, utilizing fertile soil and a conducive environment. These methods ensure the production of high quality, chemical free pepper while also promoting environmental conservation. Additionally, the average yield of black pepper in the NER is 744 kg/ha, significantly surpassing the national average of 460 kg/ha (Dinesh *et al*., 2023).

The primary breeding objectives for black pepper include achieving high yields, resistance to biotic and abiotic stresses, and favourable quality traits. Thus, activities involving the exploration of germplasm, including local cultivars, wild relatives, and related species, followed by characterization, could prove beneficial for the genetic enhancement of black pepper (Prayogya *et al*., 2020). The ICAR-Indian Institute of Spices Research (IISR), located in Kozhikode, Kerala, India, maintains the world's largest collection of black pepper germplasm and related species sourced from the centre of origin (Krishnamoorthy and Parthasarathy, 2009). Characterization and evaluation of the conserved germplasm for traits such as yield, quality, and stress resistance have led to the release of promising landraces and cultivars as popular varieties (Sasikumar *et al*., 2004).

Over the past four decades, systematic research in India, facilitated by organizations like the All India Coordinated Research Project (AICRP) on Spices and ICAR-IISR, involving hybridization, clonal selection, and open pollination from popular cultivars, has resulted in the release of superior lines of black pepper varieties (Thangaselvabal *et al*., 2008). Studies assessing released black pepper varieties from the North East region have been conducted by Deka *et al.* (2016). Despite the suitability of North East region for black pepper cultivation, its production and productivity face constraints such as the use of low yielding cultivars, limited availability of superior varieties, losses due to diseases and pests, drought, inadequate adoption of suitable agronomic practices, and a lack of quality planting materials (Krishnamoorthy and Parthasarathy, 2009). Until now, no systematic efforts have been made to enrich the varietal diversity of black pepper in North East region, except for the release of the 'Panniyur 1' variety for intercropping in arecanut plantations prevalent in the region.

To enhance varietal diversity in these regions and develop production technologies to mitigate risks faced by black pepper farmers, initiatives on collection, conservation, and characterization of black pepper accessions have been initiated in recent years by ICAR-Central Plantation Crops Research Institute (CPCRI), Research Centre, Kahikuchi, Guwahati, Assam. The present study aims to evaluate the performance of 13 local black pepper accessions under arecanut plantations at the Research farm, Kahikuchi, alongside 'Panniyur 1' as a reference variety, focusing on yield and associated traits along with quality to identify accessions suitable for cultivation in the North East region of India.

Materials and Methods

The study was conducted over a period of ten consecutive years, from 2013 to 2022, at the research farm of ICAR-CPCRI, Research Centre, located in Kahikuchi, Guwahati, Assam, India. Positioned at 20° 18' N latitude and 91° 78' E longitude, with an altitude of 50 meters above mean sea level. The farm experiences a sub-tropical climate characterized by mean maximum temperatures ranging from 15°C to 32°C and mean minimum temperatures between 8°C to 22°C, with an annual rainfall of approximately 1,500 mm. The soil composition of the experimental site is alluvial clay loam, with a pH range of 4.8 to 5.5.

Thirteen black pepper collections, bearing accession numbers IC-0599138 to IC-0599150, were included in the study. These accessions are registered at ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. The vines, aged 19 years, were sourced from farmers' fields in the Kamrup district of Assam and were supported on arecanut (*Areca catechu* L.) planted at a spacing of 2.7 m x 2.7 m.

The experiment followed a Randomized Block Design (RBD) with three replications, and within each replicate, 11 vines were selected for observations. Qualitative characteristics such as shoot tip colour, leaf margin, leaf lamina base shape, as well as lateral branch pattern, were assessed visually following the DUS (Distinctness, Uniformity, and Stability) guidelines. Additionally, quantitative traits including spike length (cm), number of berries per spike (cm), and yield were recorded as per DUS guidelines.

Quality parameters such as piperine (ASTA, 2014), essential oil (AOAC, 2005) and extraction of oleoresin was done using acetone as solvent in gravimetric method.

Young and healthy leaves from accession IC-0599150 and Panniyur 1 were used for DNA isolation by cTAB method with minor modifications (Doyle & Doyle 1990; Saghai-Maroof *et al.,* 1984). The quantity and quality of DNA was analyzed using 0.8% agarose gel and spectrophotometer readings and appropriately diluted in TE buffer. The molecular characterization was carried out using 50 random ISSR primers (Supplementary Table 1) at ICAR-Indian Institute of Spices Research (IISR), Kozhikode, Kerala. The ISSR reactions were prepared with a composition including 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9), 0.01% gelatine, 200 µM of each dNTP, 1 µM primer, 15 ng template DNA, and 0.5 unit Taq DNA polymerase in a total reaction volume of 25 µL. Amplification cycles were carried out 35 times, comprising a denaturation step at 95°C for 1 minute, annealing at the specified temperature for 1 minute, followed by extension at 70°C for 5 minutes. Post amplification, the products were loaded onto a 1.2% agarose gel containing 1x Tris-Borate EDTA and electrophoresed at 4 V/cm. The sizes of the resulting bands were determined by comparison with a 100 bp DNA ladder. Amplifications were replicated three times, and only bands consistently appearing across replicates were selected for further analysis. Each amplicon was scored as a discrete variable, with a value of 1 denoting presence and 0 indicating the absence.

Results

Qualitative traits of local black pepper accessions

The qualitative traits of 13 local black pepper accessions were evaluated and summarized in Table 1. These accessions demonstrated notable diversity in morphological characteristics. Shoot tip color varied from dark purple in most accessions to light purple in IC-0599145 and light green in IC-0599147. Leaf margins were predominantly even across accessions, though leaf base shape differed, with most accessions showing a round leaf base, except for IC-0599148 and IC-0599149, which exhibited an acute leaf base.

The branching pattern further highlighted the genetic diversity among the accessions. While many accessions, such as IC-0599140, IC-0599142, IC-0599143, and IC-0599145, demonstrated a horizontal branch pattern, others, like IC-0599138, IC-0599139, IC-0599141 and IC-0599144 exhibited a semi-erect branching pattern. These traits are presented in Table 1 and can be visualized in Figure 1, showing the phenotypic variability in the accessions.

Spike length and berry count

Spike lengths were categorized as either short or medium across the accessions. Most accessions produced short spikes, but IC-0599145, IC-0599150, and Panniyur 1 had medium-length spikes. The distribution of berry count also demonstrated genetic variation. IC-0599147 was classified as having a medium number of berries, while the remaining accessions, including IC-0599150 and Panniyur 1, exhibited many berries per spike, which likely contributes to their higher yields.

These findings suggest that spike length and berry count might influence the productivity of black pepper accessions, correlating with the observed yield performance in subsequent years. The combination of longer spikes and a higher number of berries may offer a competitive advantage for pepper yields in accessions like IC-0599150.

Phytochemical composition

Phytochemical analyses revealed significant differences among the accessions, particularly in the levels of piperine, essential oils, and oleoresin. As shown in Table 2, the piperine content ranged from 3.67% in Panniyur 1 to a high of 5.63% in IC-0599143. The highest essential oil content was observed in IC-0599139 and IC-0599142 (3.70%), while IC-0599145 had the lowest (2.03%).

Oleoresin content, an important trait for marketability, also exhibited variability across accessions. IC-0599142 recorded the highest concentration at 9.60%, whereas IC-0599145 showed the lowest (7.08%). These differences in phytochemical composition suggest that certain accessions like IC-0599142 and IC-0599143 are of particular interest for high quality spice production due to their superior piperine and oleoresin content.

Yield parameters of pepper genotypes

Yield data collected over a ten-year period (2014-2023) displayed substantial variation across the accessions (Figure 2 and Figure 3). IC-0599150 demonstrated the highest mean yield of 6.662 kg/plant, while IC-0599145 followed closely with an average yield of 6.356 kg/plant. Panniyur 1, a standard variety used as a reference, maintained relatively stable yields, averaging 4.539 kg/plant.

Interestingly, higher yields were consistently observed in the years 2018 and 2020, possibly due to favorable environmental conditions or better management practices. However, the yields in 2016 and 2017 were notably lower, indicating the susceptibility of these genotypes to environmental fluctuations. Overall, the data suggests that IC-0599150 and IC-0599145 are superior in terms of yield potential under varying environmental conditions, making them promising candidates for cultivation.

Clustering of pepper genotypes

Based on both quantitative and qualitative traits, the pepper accessions were grouped into four distinct clusters (Table 3 and Figure 4). Cluster 1 contained high-yielding genotypes IC-0599145 and IC-0599150, characterized by medium spike length and many berries, in addition to having high piperine content. IC-0599138 was placed in Cluster 2, identified by its unique trait combination and lower yield compared to other genotypes.

Panniyur 1 and IC-0599147 were grouped into Cluster 3, exhibiting moderate yields and phytochemical composition. Cluster 4 comprised the remaining nine accessions, which showed a broad range of traits but generally lower yield potential compared to those in Cluster 1. This clustering highlights the genetic diversity within the local pepper accessions and emphasizes the potential for selecting specific genotypes based on desired traits.

Molecular diversity

To complement the phenotypic and yield data, DNA fingerprinting using 50 ISSR markers was employed to assess the genetic distinctiveness between the accessions. Notably, IC-0599150 showed the presence of the band ISSR-011000 and the absence of ISSR-01510, distinguishing it from Panniyur 1 (Figure 5). This molecular data aligns with the clustering results, further reinforcing the genetic uniqueness of IC-0599150 and its potential as a high-performing genotype.

The molecular analysis confirms that IC-0599150 is genetically distinct from other accessions, supporting the observed differences in yield and phytochemical traits. These findings suggest that IC-0599150 could be a valuable candidate for breeding programs aimed at improving yield and quality in black pepper.

Discussion

The qualitative traits observed in local black pepper accessions, such as shoot tip coloration, leaf margin, and lateral branch patterns, provide valuable insights into the genetic diversity of these accessions. These traits are vital for distinguishing between accessions, which is crucial for selecting varieties suited to specific growing conditions or market demands. The classification of spike lengths and berry counts is consistent with prior research by Shango *et al.* (2021), emphasizing the role of such traits in germplasm characterization and breeding efforts.

The significant variability in phytochemical composition, particularly piperine, essential oils, and oleoresins, aligns with findings by Ghosh and Chatterjee (2023), who highlighted the importance of these compounds in enhancing the culinary and medicinal value of black pepper. Accessions with high piperine content, such as IC-0599143, hold promise for breeding programs aimed at enhancing pungency, while those with high essential oil content could be targeted for aroma and flavor improvement in the food industry. The variability in oleoresin content also points to opportunities for improving pepper accessions for oleoresin extraction, as noted by Ravindran & Pillai (2017).

The yield analysis over a ten-year period demonstrated significant fluctuations in productivity, which is likely influenced by annual climatic conditions, as suggested by Jamal *et al.* (2009). Genotypes such as IC-0599150 and IC-0599145, which consistently exhibited high yields, are prime candidates for large-scale cultivation and breeding programs aimed at yield improvement. Panniyur 1, although not the highest yielder, remains a reliable benchmark due to its stable performance across years. This underscores the importance of selecting genotypes with both high yield potential and stability, a key factor highlighted in recent studies on multi-trait selection in crop improvement (Rao *et al.*, 2020; Kavya *et al.*, 2020).

The clustering analysis further emphasizes the genetic diversity within the black pepper accessions, which is crucial for breeding programs. The placement of IC-0599150 in Cluster 1, distinct from Panniyur 1 in Cluster 3, reflects their genetic and agronomic differences, supporting the notion of IC-0599150 as a superior candidate for varietal release. The molecular diversity analysis using ISSR markers reinforces this distinction, with Sharma *et al.* (2023) and Gupta & Singh (2022) underscoring the value of molecular markers in differentiating and selecting genotypes for breeding.

Conclusion

Superior yield performance, phytochemical richness, and genetic distinctiveness of IC-0599150 position it as a highly promising candidate for breeding programs and commercial cultivation. The successful identification of unique molecular markers further enhances its potential for adoption as a new high-yield variety, contributing to the advancement of black pepper cultivation and genetic improvement efforts.

Table 1 : Qualitative and quantitative traits of local black pepper accessions

Accessions	Shoot tip colour	Leaf base shape	Lateral branch pattern	Leaf margin	Spike length (cm)	No. of berries /spike	Mean fresh Yield (kg/plant)	Mean dry Yield (kg/plant)
IC-0599138	Dark purple	Round	Semi erect	Even	9.49	56.07	5.15	1.76*
IC-0599139	Dark purple	Round	Semi erect	Even	9.19	54.21	5.028	1.39
IC-0599140	Dark purple	Round	Horizontal	Even	9.72	59.45	5.421	1.70*
IC-0599141	Dark purple	Round	Semi erect	Even	9.57	53.43	4.576	1.23
IC-0599142	Dark purple	Round	Horizontal	Even	9.32	57.37	3.811	0.99
IC-0599143	Dark purple	Round	Horizontal	Even	9.16	57.36	5.495*	1.79*
IC-0599144	Dark purple	Round	Semi erect	Even	8.31	50.63	5.306	1.17
IC-0599145	Light purple	Round	Horizontal	Even	12.01*	64.64*	6.356*	2.01*
IC-0599146	Dark purple	Round	Horizontal	Even	8.74	55.80	4.832	1.25
IC-0599147	Light green	Round	Horizontal	Even	8.72	47.28	4.096	0.86
IC-0599148	Dark purple	Acute	Horizontal	Even	8.35	52.22	3.719	1.08
IC-0599149	Dark purple	Acute	Horizontal	Even	8.68	50.12	5.11	1.65
IC-0599150	Dark purple	Round	Horizontal	Even	11.59*	80.69*	6.602*	2.14*
Panniyur 1	Light green	Round	Horizontal	Even	11.33*	52.58	4.539	1.49
Mean					9.584	56.561	5.003	1.465
CV					12.551	14.470	16.860	26.568
SD					1.203	8.184	0.843	0.389
SE					0.322	2.187	0.225	0.104

Table 2 : Phyto chemical components of black pepper accessions

Accessions	Moisture (%)	Piperine (%)	Essential oil (%)	Oleoresin (%)
IC-0599138	14.50	4.95	3.10	8.81
IC-0599139	14.30	5.03	3.70	8.94
IC-0599140	13.40	5.46	3.56	9.50
IC-0599141	14.30	5.60	3.56	9.01
IC-0599142	13.90	5.60	3.70	9.60
IC-0599143	13.96	5.63	3.20	9.28
IC-0599144	14.56	5.54	3.63	9.26
IC-0599145	13.63	3.96	2.03	7.08
IC-0599146	14.03	5.49	3.56	9.35
IC-0599147	14.20	5.24	3.30	8.58
IC-0599148	13.66	5.57	3.43	9.10
IC-0599149	13.00	5.22	3.30	8.79
IC-0599150	14.43	5.10	3.43	9.36
Panniyur 1	15.50	3.67	2.70	7.84
SEm (+/-)	0.31	0.13	0.12	0.24
CD (0.05%)	0.90	0.43	0.35	0.72

Table 3 : Hierarchical clustering of pepper genotypes based on qualitative and quantitative data

Clusters	No. of Accessions	Name of the Accessions
Cluster 1	2	IC-0599145, IC-0599150
Cluster 2	1	IC-0599138
Cluste	2	IC-0599147, Panniyur 1

r 3		
Cluster 4	9	IC-0599139, IC-0599140, IC-0599141, IC-0599142, IC-0599143, IC-0599144, IC-0599146, IC-0599148, IC-0599149

Fig. 1 : Variation in leaf size, shape and leaf margin in black pepper genotypes

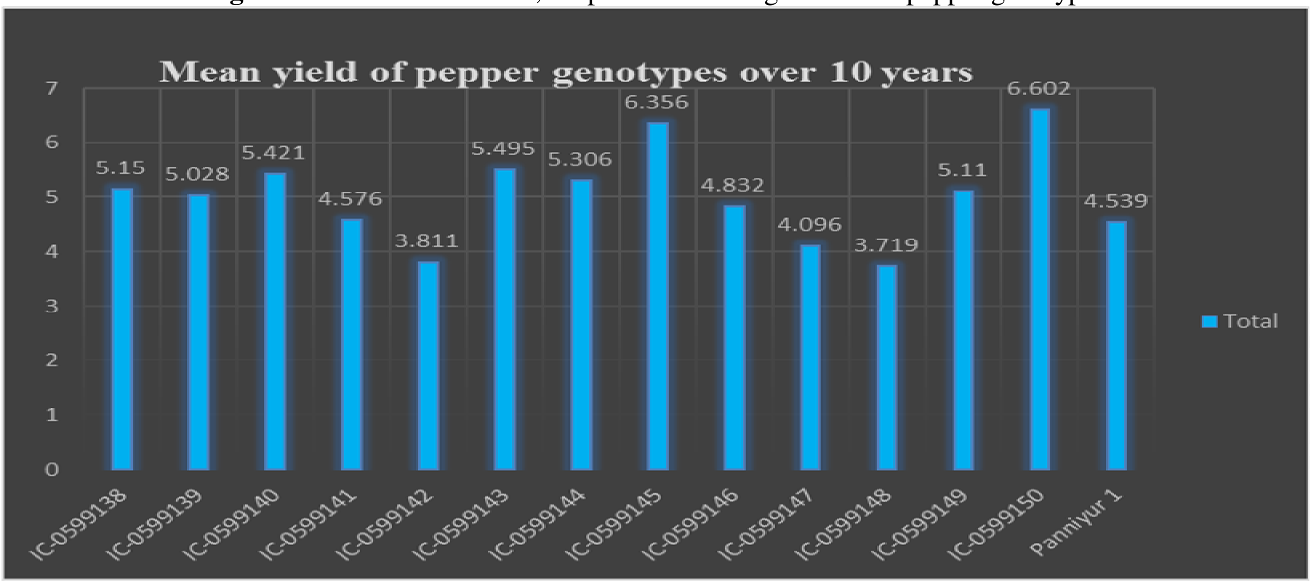

Fig. 2 : Mean yield of black pepper genotypes based on ten year data.

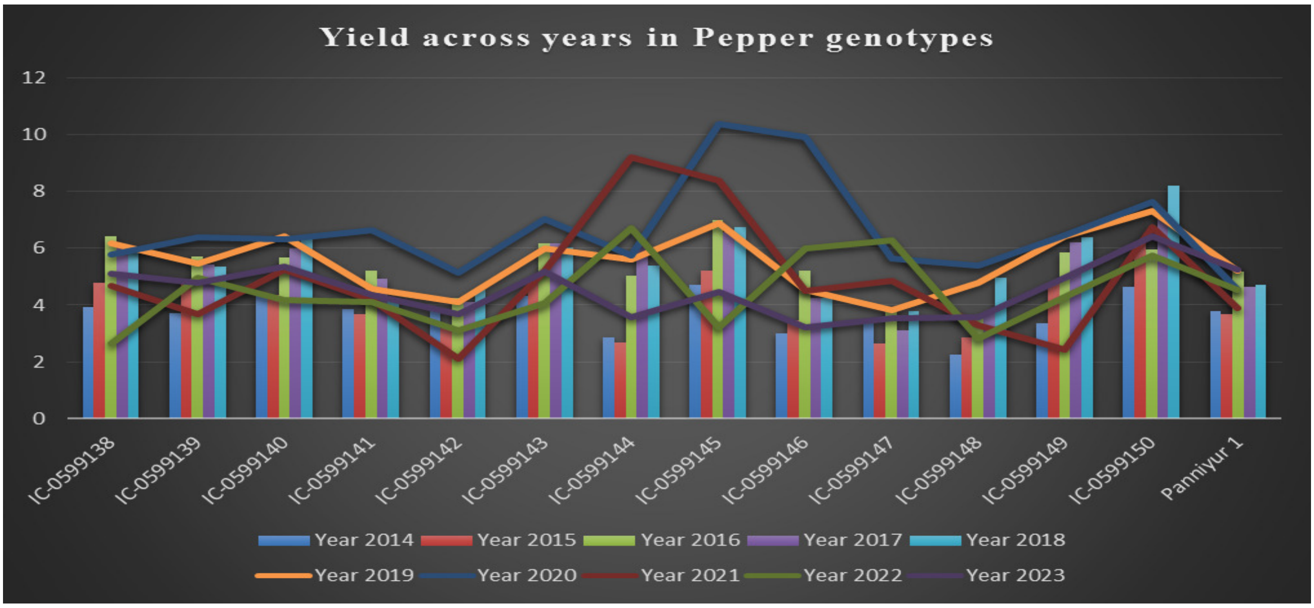

Fig. 3 : Yield of black pepper genotypes across years

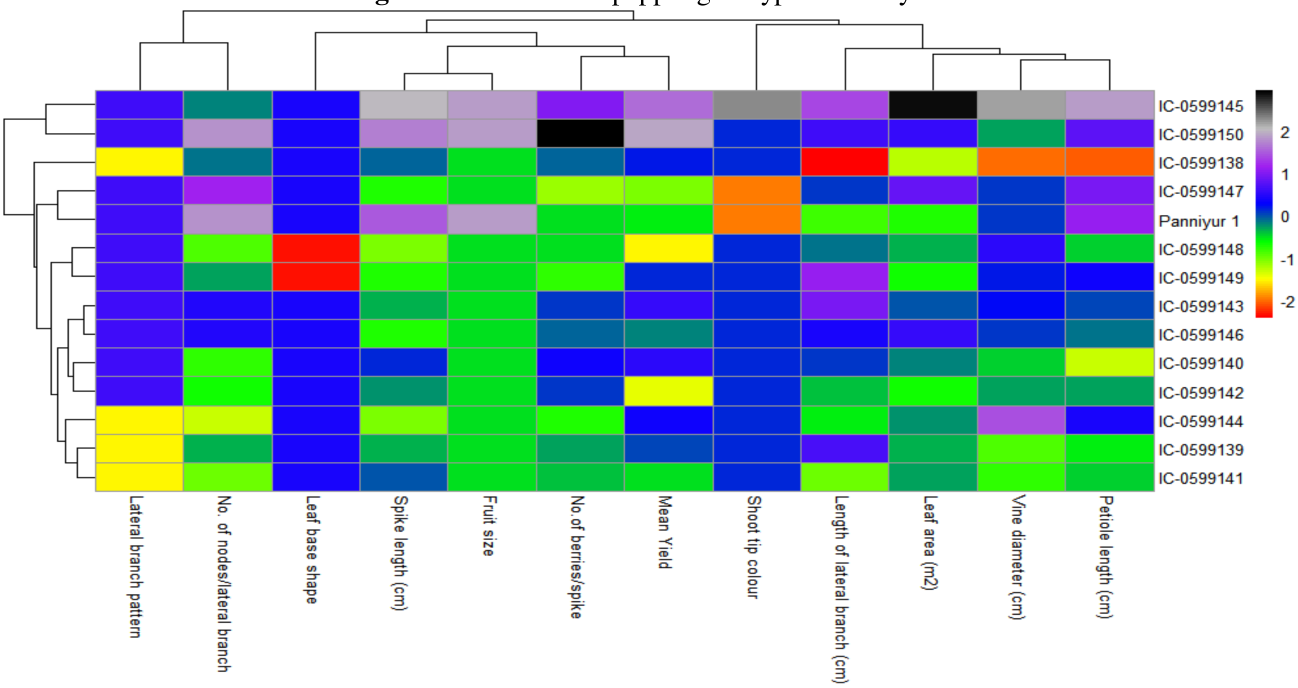

Fig. 4 : Hierarchical heatmap clustering of pepper genotypes based on quality and quantitative data

Fig. 5 : ISSR-01 markers with polymorphism for Panniyur 1 and IC-0599150 Primer ISSR-01; L-ladder (Gene Ruler^{TM} 1 Kb plus); Lane-1 Panniyur-1, Lane-2 KKHC-13. Red arrow indicates the presence of unique bands.

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Disclosure statement

The authors report there are no competing interests to declare.

Data availability statement

The authors confirm that the data supporting the results of this study are available in the article. We are therefore at your disposal for any additional needs.

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