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Phenotypic Variability among Chilli Germplasms Using Shannon-Weiner Index (H')

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ABSTRACT

The investigation was conducted during the *rabi* season (September 2019 to February 2020) of 2019 to 2020 at the research field of All India Co-ordinated Vegetable Crops, located at Bidhan Chandra Krishi Viswavidyalaya in Kalyani, Nadia, West Bengal, India. The aim of the study was to examine the characteristics of twenty-six chilli genotypes across twelve qualitative traits. The investigation unveiled a broad range of phenotypic variability using Randomized Block Design in three replications. Among the assessed traits, leaf density, leaf shape, fruit shape, plant habit, fruit curvature, and anther colour exhibited the most extensive variations among the genotypes. Conversely, the traits with the least diversity values, all falling below the overall mean, included fruit surface (0.28%), leaf colour (0.42%), stem pubescence (0.42%), stem colour (0.42%), anthocyanin colouration at nodes (0.48%), and leaf pubescence (0.48%). The Shannon-Weiner Diversity Index (H') ranged from 0.28 to 1.07. Of the traits evaluated, leaf density displayed the highest diversity index (1.07), followed by leaf shape (1.05), fruit shape (1.01), plant habit (0.86), fruit curvature (0.69), and anther colour (0.58). The obtained Shannon-Weiner Diversity Index, with an average value of 64.66%, confirmed the presence of significant diversity within the examined chilli genotypes. The results suggest that the indirect selection of chilli parent plants based on a range of morphological characteristics can offer advantages in the management of both biotic and abiotic stresses. The information holds significant value for effective pre-breeding, management, and utilization in crop improvement initiatives, in addition to augmenting the genetic potential of the crop.

KEYWORDS: Chilli, characterization, frequency distribution, shannon-weiner diversity index

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1. INTRODUCTION

In India, the chilli pepper (*Capsicum annuum* Linn.) holds a prominent position among Solanaceous vegetables crops. India stands as the world's leading producer, exporter, and consumer of chilli peppers (Halder et al., 2021). In addition to the traditional use of capsicum as fresh produce, its demand extends to various processed forms such as sauces, pickles, canned products, dried spices, and industrial extracts (Meghvansi et al., 2010). The significance of capsicum is further underscored by its nutritional value, including ascorbic acid (vitamin C), carotenoids (Pro-vitamin A), tocopherols (vitamin E), flavonoids, and capsaicinoids (Bal et al., 2022). Moreover, it finds utility in non-food sectors, notably in defense as ethnobotanical products (Tiwari and Srinivasan, 2013).

The growing importance of chilli peppers necessitates an increase in yield, which can be achieved through the collection and characterization of different genotypes (Rani and Kumari 2016). Various morphological characteristics, including plant height, fruit shape, fruit weight, and flower color, are employed for the grouping and categorization of diverse chilli genotypes (Fonseca et al., 2008). Phenotypic characterization is a crucial aspect for classifying germplasm in any characterization program, as emphasized by Tyler et al. (2014). The critical procedures of maintaining a dynamic foundation for exploring genetic variability in chilli include morphological characterization, assessing genetic diversity, and documenting in gene banks (Lannes et al., 2007; Bal et al., 2022; Paran and van der Knaap, 2007; Bal et al., 2023). Genebanks installed as genetic repositories are often affected by mismanagement, consisting of a series of mistakes, such as inadequate storage, duplicate accessions and loss of variability due to genetic erosion (Mallikarjuna et al., 2011). In this sense, the characterization of the accessions conserved in genebanks is understood as essential for the species' conservation and use in plant breeding programs (Bal et al., 2024; Nimmakayala et al., 2014). Thus, conservation of this diversity in genebanks is essential, given the genetic erosion resulting from the destruction of natural habitats, the replacement of local varieties with improved cultivars, and the agricultural abandonment of pepper-producing smallholders (Sudhakar et al., 2004). This information is crucial for identifying parents with strong combining potential and obtaining superior genotypes (Bal et al., 2019). Despite numerous advancements in biotechnology and bioinformatic tools, breeders still commonly base their selection of parents on the phenotypic performance related to yield components, vegetative and reproductive cycles, and resistance to pests and diseases (Kumar and Sarbhoy, 2005). Germplasm conservation serves as the foundational material that scientists can harness to enhance crop productivity and diversify production systems (Schreinemachers et al., 2014). Proper evaluation of accessions for conservation in gene banks is essential, ensuring their availability for breeding programs (Sudre et al., 2010). Plant breeders can enhance the development of breeding populations by incorporating genetic similarity information alongside phenotypic data (Kumar and Choudhary, 2014). Therefore, the characterization of germplasm is crucial for the conservation and effective utilization of plant genetic resources (Thul et al., 2012).

The genetic material at the disposal of plant breeders is a pool of genes that must be identified, isolated, and integrated into the genome of a new genetic entity that exhibits a unique and desirable phenotype (Tiwari et al., 2021). Transforming this raw genetic material into high-yielding varieties involves a process of domestication, refinement, and maintenance, typically carried out by local farmers (Rabara et al., 2014). The characterization of these farmer-developed varieties may uncover valuable genes for revolutionizing plant breeding (Fonseca et al., 2008). The rich gene pool and meticulous selection processes employed in varietal development, coupled with molecular characterization, provide valuable insights into the future utilization of existing genetic diversity (Sivasubramanian et al., 2014).

In accordance with the Protection of Plant Varieties and Farmers' Rights Act (PPV&FRA) of 2001, the protection of these varieties hinges on their Distinctiveness, Uniformity, and Stability (DUS). The research presented in this context focuses on phenotypically assessing diversity since diverse genotypes serve as an excellent resource for creating new and improved crop varieties.

2. MATERIALS AND METHODS

The experiment was performed during the Rabi season (September-February) of 2019 to 2020 in the research field of All India Coordinated Vegetable Crops, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal located at 23.5° N latitude and 89° E longitude at an elevation of 9.75m above the mean sea level. Twenty-six advanced lines/varieties/accessions of chilli collected from BCKV, West Bengal, GBPUA&T, Pantnagar, MPKV, Rahuri and NBPGR, New Delhi constituted the plant materials for this study. The genotypes were grown in Randomized Block Design with three replications. Phenotypic observations were recorded from 10 randomly selected plants of each plot in each replication as per documented descriptors of chilli (Srivastava, 2001). The characters studied were represented in Table 1. Shannon-Weiner Diversity Index (H') was used to calculate the phenotypic diversity. Shannon-Weiner Diversity Index

Table 1: Morphological traits taken under study

Plant traits	Fruit traits
Plant habit	Fruit shape
Leaf shape	Fruit surface
Leaf colour	Anther colour
Stem pubescence	Fruit curvature
Anthocyanin colouration at nodes	
Stem colour	
Leaf density	
Leaf pubescence	

(Shannon, 1948) is a commonly used diversity index that takes into account both the abundance and evenness of a character present in the population. It is explained by the following formula:

Shannon Diversity, $(H') = -\sum_{i=1}^{s} p_i \ln p_i$

Where, the p is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), ln is the natural log, $\sum$ is the sum of the calculations, and 's' is the number of species.

3. RESULTS AND DISCUSSION

Through the ages, visual observations (scoring) have served as a tool to assess genetic diversity within the gene pool. The characterization of twenty-six genotypes articulated variability of the traits studied. As many as twelve qualitative characters traits^{-1} viz., plant habit, leaf Shape, leaf colour, stem pubescence, anthocyanin colouration at nodes, stem colour, leaf density, leaf pubescence, fruit shape, fruit surface, anther colour and fruit curvature were recorded in twenty-six chilli genotypes as per the minimal descriptors of NBPGR to characterize the present diversity of chilli (Table 2 and 3). Frequency distribution of twenty-six genotypes according to different characters was worked out and were presented (Table 4).

Table 2: Morphological characterization depicting the predominate state for each descriptor studied

Genotypes	Plant habit	Leaf shape	Leaf colour	Stem pubescence	Anthocyanin colouration at nodes	Stem colour
Chinese Bona	Spreading	Lanceolate	Green	Present	Absent	Green
Pant C 1	Upright	Ovate	Green	Absent	Present	Green
Bidhan Chilli 4	Semi-upright	Lanceolate	Green	Present	Present	Green
Chilli 38-Ragi	Semi-upright	Lanceolate	Green	Absent	Present	Green
Srinagar	Semi-upright	Lanceolate	Green with purple tinge	Present	Present	Green with purple tinge
BCC 1	Semi-upright	Lanceolate	Green	Present	Present	Green
G-4 Ziya Chibli	Semi-upright	Broad elliptic	Green	Present	Present	Green
Phule Jyoti Garima	Semi-upright	Lanceolate	Green	Absent	Absent	Green
Bangla Magura	Semi-upright	Lanceolate	Green	Present	Present	Green
Akashi Lanka	Upright	Broad elliptic	Green with purple tinge	Absent	Present	Green with purple tinge
Chilli IR-8	Semi-upright	Ovate	Green	Present	Present	Green
Dhani Lanka	Spreading	Ovate	Green	Present	Absent	Green
Suryamukhi	Semi-upright	Lanceolate	Green with purple tinge	Present	Present	Green with purple tinge
G-4	Upright	Broad elliptic	Green	Present	Present	Green
BCC-30	Semi-upright	Lanceolate	Green	Present	Present	Green
BCC-25	Semi-upright	Ovate	Green	Present	Present	Green
EC-382175	Semi-upright	Ovate	Green	Present	Absent	Green
EC390029	Semi-upright	Broad elliptic	Green	Present	Present	Green
IC-255916	Semi-upright	Ovate	Green	Present	Present	Green
IC-255944	Semi-upright	Broad elliptic	Green	Present	Present	Green
IC-383072	Semi-upright	Broad elliptic	Green	Present	Present	Green

Table 2: Continue...

Genotypes	Plant habit	Leaf shape	Leaf colour	Stem pubescence	Anthocyanin colouration at nodes	Stem colour
IC-570408	Spreading	Lanceolate	Green with purple tinge	Present	Present	Green with purple tinge
NIC-19966	Semi-upright	Ovate	Green	Present	Absent	Green
NIC-19967	Semi-upright	Ovate	Green	Present	Present	Green
PBC-613	Upright	Lanceolate	Green	Present	Present	Green
PBC-824	upright	lanceolate	Green	Present	Present	Green

Table 3: Morphological characterization depicting the predominate state for each descriptor studied

Genotypes	Leaf density	Leaf pubescence	Fruit shape	Fruit surface	Anther colour	Fruit curvature
Chinese Bona	Sparse	Sparse	Horn shaped	Smooth	Pale blue	Present
Pant C 1	Dense	Sparse	Moderately triangular	Rough	Pale blue	Absent
Bidhan Chilli 4	Dense	Sparse	Narrowly triangular	Smooth	Pale blue	Absent
Chilli 38-Ragi	Dense	Sparse	Narrowly triangular	Smooth	Pale blue	Present
Srinagar	Sparse	Sparse	Horn shaped	Smooth	Pale blue	Present
BCC 1	Intermediate	Sparse	Moderately triangular	Smooth	Pale blue	Absent
G-4 Ziya Chibli	Intermediate	Sparse	Narrowly triangular	Smooth	Purple	Present
Phule Jyoti Garima	Dense	Intermediate	Moderately triangular	Smooth	Pale blue	Absent
Bangla Magura	Sparse	Intermediate	Narrowly triangular	Smooth	Pale blue	Present
Akashi Lanka	Intermediate	Sparse	Moderately triangular	Rough	Purple	Absent
Chilli IR-8	Dense	Sparse	Horn shaped	Smooth	Pale blue	Present
Dhani Lanka	Sparse	Sparse	Narrowly triangular	Smooth	Pale blue	Present
Suryamukhi	Sparse	Intermediate	Narrowly triangular	Smooth	Pale blue	Absent
G-4	Intermediate	Sparse	Horn shaped	Smooth	Pale blue	Absent
BCC-30	Intermediate	Sparse	Narrowly triangular	Smooth	Pale blue	Present
BCC-25	Intermediate	Sparse	Narrowly triangular	Smooth	Pale blue	Absent
EC-382175	Sparse	Sparse	Moderately triangular	Smooth	Purple	Present
EC390029	Sparse	Intermediate	Moderately triangular	Smooth	Pale blue	Present
IC-255916	Intermediate	Sparse	Moderately triangular	Smooth	Purple	Present
IC-255944	Intermediate	Intermediate	Moderately triangular	Smooth	Pale blue	Absent
IC-383072	Sparse	Sparse	Moderately triangular	Smooth	Pale blue	Present
IC-570408	Dense	Sparse	Narrowly triangular	Smooth	Purple	present
NIC-19966	Sparse	Sparse	Moderately triangular	Smooth	Purple	Absent
NIC-19967	Sparse	Sparse	Moderately triangular	Smooth	Pale blue	Absent
PBC-613	Intermediate	Sparse	Narrowly triangular	Smooth	Pale blue	Present
PBC-824	Intermediate	Sparse	Moderately triangular	Smooth	Purple	Absent

3.1. Plant habit

In the current study, three distinct types of plant habits were identified: spreading, upright, and semi-upright (Table 3). The prevailing plant habit observed was semi-upright, accounting for 69.23% of the cases. Specifically, genotypes Chinese Bona, Dhani Lanka, and IC-570408 showcased a spreading plant habit (11.5%), whereas genotypes Pant C 1, Akashi Lanka, G-4, PBC-613, and PBC-824 exhibited an upright plant habit (19.2%).

Table 4: Frequency distribution and H' index for different characters in chilli genotypes

Characters/ Traits	Specifications	No. of genotypes	Percentage of genotypes (%)	H' index
Plant habit	Spreading	3	11.5	0.86
	Upright	5	19.2	
	Semi-upright	18	69.23	
Leaf shape	Lanceolate	12	46.15	1.05
	Ovate	8	30.76	
	Broad-elliptic	6	26.92	
leaf color	Green	22	84.62	0.42
	Green with purple tinge	4	15.38	
Stem pubescence	Present	22	84.61	0.42
	Absent	4	15.38	
Anthocyanin coloration at nodes	Present	21	80.77	0.48
	Absent	5	19.23	
Stem color	Green	22	84.61	0.42
	Green with purple tinge	4	15.38	
leaf density	Sparse	10	38.46	1.07
	Intermediate	10	38.46	
	Dense	6	23.07	
Leaf pubescence	Sparse	21	80.76	0.48
	Intermediate	5	19.23	
Fruit shape	Horn shaped	4	15.38	1.01
	Moderately triangular	12	46.15	
	Narrowly triangular	10	38.46	
Fruit surface	Smooth	24	92.31	0.28
	Rough	2	7.69	
Anther colour	Purple	7	26.92	0.58
	Pale blue	19	73.08	
Fruit curvature	Present	14	53.85	0.69
	Absent	12	46.15	
Overall mean of H'				0.6466

3.2. Leaf shape

Among the 26 genotypes examined, 46.15% exhibited a lanceolate leaf shape, while 30.76% had an ovate leaf shape, and 26.92% displayed a broad-elliptic leaf shape.

3.3. Leaf colour

The leaf colour of 26 genotypes was classified into two categories: green and green with purple tinge. Among these, Srinagar, Akashi Lanka, Suryamukhi, and IC-570408 displayed a green colour with tinge of purple, while the remaining genotypes featured a purely green leaf colour, accounting for 84.62% of the total.

3.4. Stem pubescence

The majority of genotypes (84.61%) exhibited a prominent presence of stem pubescence, while four genotypes, namely, Pant C 1, Chilli 38-Ragi, Phule Jyoti Garima, and Akashi Lanka, were observed to lack it.

3.5. Anthocyanin colouration at nodes

Among the twenty-six genotypes, 80.77% exhibited anthocyanin colouration at the nodes of chilli plants, while 19.23% of the genotypes lacked this trait.

3.6. Stem colour

Two stem colour variations were identified, namely, green and green with purple tinge. Among the genotypes, Srinagar, Akashi Lanka, Suryamukhi, and IC-570408 exhibited the green with a purple tinge stem colour (15.38%), while the remaining genotypes displayed a green stem colour (84.61%).

3.7. Leaf density

The leaf density of twenty-six genotypes was classified into three categories: sparse, intermediate, and dense (Table 3). Among these genotypes, 38.46% displayed sparse leaf density, while 38.46% had intermediate leaf density, and 23.07% exhibited a dense leaf density.

3.8. Leaf pubescence

The presence of leaf pubescence was predominant in the majority of genotypes, accounting for 80.76%, while approximately 19.23% of genotypes displayed sparse leaf pubescence.

3.9. Fruit shape

Among the twenty-six genotypes under examination, 46.15% displayed a fruit shape that was moderately triangular, while 15.38% and 38.46% exhibited fruit shapes characterized as horn-shaped and narrowly triangular, respectively.

3.10. Fruit surface

The genotypes displayed two distinct characteristics in terms of fruit surface at harvest maturity: smooth and wrinkled.

In this study, 7.69% of the genotypes featured a rough fruit surface, while 92.31% of the genotypes had a smooth fruit surface.

3.11. Anther colour

Anthers were observed to have varied colours, including purple and pale blue. Among the genotypes, 73.08% exhibited a pale blue colour for their anthers, while 26.92% displayed blue anthers.

3.12. Fruit curvature

In this study, 53.85% of the genotypes displayed fruit curvature, while the remaining 46.15% did not exhibit any curvature in their fruits.

Frequency distribution is a systematic method of organizing a dataset, arranging it from the lowest to the highest values, while indicating the number of occurrences (Frequency) for each value or value range. These frequency distributions were utilized to compute the Shannon-Weiner diversity index (H') for each trait, a concept initially proposed by Hennick and Zeven in 1991. The Shannon diversity index (H') was calculated to assess phenotypic diversity among the traits used in the study, highlighting its crucial role in evaluating the genetic resource collections within a gene bank. This index facilitates the analysis of the frequency distribution of traits within a given population. The Shannon-Weiner Diversity Index for twelve different traits in twenty-six chilli genotypes, and the results were displayed in Table 3.

Crop biodiversity is characterized by both allelic evenness and allelic richness (Bal et al., 2020). When conducting a morphological assessment, descriptors and their states correspond to loci and alleles, and this relationship is crucial when applying the Shannon-Weiner Diversity Index (H'). In this context, allelic evenness was quantified using the Shannon-Weiner Diversity Index, while allelic richness was determined by counting the descriptor states for each descriptor, irrespective of individual frequencies. Richness indicates the number of genotypes present in a specific area, whereas evenness reflects the relative abundance of each genotype. A higher value signifies greater species diversity (Belay and Tsehaye, 2020).

In the current investigation, the Shannon-Weiner Diversity Index (H') exhibited a range from 0.28 to 1.07. Among the traits assessed, leaf density displayed the highest diversity (1.07), followed by leaf shape (1.05), fruit shape (1.01), plant habit (0.86), fruit curvature (0.69), and anther colour (0.58). A significant Shannon-Weiner Diversity Index, with an average value of 64.66%, was achieved, affirming the presence of diversity within the studied chilli genotypes. This result aligns with the findings of Shimeles (2018), who identified a higher level of diversity, ranging from 0.65 to 0.98, among hot pepper genotypes in terms of quality traits. This diversity was observed in regions including Bale, Halaba, Assossa, Abshge, and Marko in Ethiopia.

In this study, the traits that exhibited the most extensive variations among the genotypes were leaf density, leaf shape, fruit shape, plant habit, fruit curvature, and anther colour. The traits with the lowest diversity values, all falling below the overall mean, included fruit surface (0.28%), leaf colour (0.42%), stem pubescence (0.42%), stem colour (0.42%), anthocyanin colouration at nodes (0.48%), and leaf pubescence (0.48%). Nsabiyera et al. (2013) documented in their studies the frequency distribution and Shannon-Weiner diversity index, revealing significant divergence among qualitative traits within their hot pepper collections. The Shannon-Weiner Diversity Index (H') values typically fall within the range of 0 to approximately 4.6. A value close to 0 suggests that all genotypes in the sample are identical, while a value near 4.6 indicates an even distribution of individuals among chilli genotypes. According to Hennink and Zeven (1991), a low H' value signifies an uneven and unbalanced frequency distribution, indicating a lack of diversity in a particular trait for any given genotype. Conversely, a higher H' value suggests the presence of variability and diversity within that trait. Moreover, values below the overall mean indicate an unbalanced frequency distribution and a lack of diversity for the traits.

Such comprehensive analysis of the characteristic traits of all twenty-six chilli genotypes was conducted, taking into consideration the primary species-defining features of the *Capsicum* genus, as outlined by Bosland and Votava (1999). It was conclusively determined that the majority of the genotypes belonged to *Capsicum annuum* L., while seven genotypes, characterized by the presence of purple anthers, were indeed *Capsicum frutescens*. Nevertheless, a more in-depth morphological assessment and the use of molecular markers are required to definitively confirm their classification within distinct species.

4. CONCLUSION

The study unveiled phenotypic variations in twenty-six chilli genotypes, pivotal for pre-breeding, management, and crop enhancement. Findings suggested that indirect selection of parent plants based on morphological traits aided in addressing biotic and abiotic stresses. Attributes like leaf pubescence and drooping fruit habit were found advantageous. Various morphological traits, including leaf shape, pubescence, stem pubescence, fruit shape, and anthocyanin colouration at nodes, were utilised in breeding programmes. The study backed ongoing breeding efforts for high-yield, climate-resilient varieties, stressing alternative varietal selections to enrich agricultural biodiversity and meet user preferences.

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