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Quantification of capsaicin content and pungency variations across Dalle Khursani accessions, a polyploid capsicum landrace from the Darjeeling-Sikkim Himalayas, India

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Abstract

Dalle Khursani, a unique landrace of *Capsicum* sp specific to the Darjeeling-Sikkim Himalaya in India, is highly valued for its pungency. The present study evaluated the capsaicin content and pungency levels of seven accessions collected from diverse altitudinal locations. The concentration of capsaicin varied between 19.05 mg/g to 28.54 mg/g, with corresponding Scoville Heat Units (SHU) ranging from 304,863 to 456,636. The results indicated the presence of significant variations for pungency within the accessions of Dalle Khursani, which may be attributed to genetic factors with the micro-climatic factors, soil composition and water availability contributing to pungency. This study fills critical research gaps with the first quantitative evaluation of capsaicin content and pungency and identified accessions such as DK-02 and DK-03 as superior in capsaicin content, making them potential candidates for further selection and genetic improvement.

Key words: Dalle Khursani, polyploid capsicum, capsaicin content, pungency, HPLC

Introduction

Capsicum is one of the most consumed spices globally and has been cultivated for thousands of years for its culinary, medicinal, and economic value. Its characteristic pungency is attributed to capsaicin, a bioactive alkaloid exclusive to the genus *Capsicum*. Capsaicin is synthesised through a complex biochemical pathway involving several key genes, such as Pun1 and Csy1, which play critical roles in capsaicinoid biosynthesis. Environmental factors, including light intensity, temperature, and agronomic conditions, also significantly influence capsaicinoid metabolism, resulting in considerable variability in pungency across chilli genotypes.

Landraces, defined as genetically diverse plant populations adapted to local agro-climatic conditions are named, selected, and preserved by traditional farmers to meet their social, economic, and ecological needs (Teshome *et al.,* 1997). Dalle Khursani, a unique chilli landrace indigenous to the Darjeeling-Sikkim Himalayan region, exemplifies this concept. Known for its intense pungency and distinct flavour, this landrace has been granted Geographical Indication (GI) status, highlighting its unique regional identity. Its reported polyploid nature (Jha *et al.*, 2017) enhances its adaptability to the challenging agro-climatic conditions of this region, characterised by high altitudes, fluctuating temperatures, and limited water availability. Polyploids, in general, exhibit superior resilience compared to their diploid counterparts, making Dalle Khursani particularly well-suited for cultivation in these marginal environments.

The pungency of chilli peppers is directly linked to capsaicin and dihydrocapsaicin, which together constitute ~90% of the total capsaicinoids (Kosuge and Furuta, 1970). Variations in capsaicin content among chilli accessions are known to be influenced by genetic and environmental factors (Zewdie and Bosland, 2000). For instance, the niche environment of Dalle Khursani, with its cool and humid climate and specific temperature ranges (11.0°C–20.7°C) during the crop period (Verma *et al.*, 2024), plays a significant role in regulating capsaicinoid metabolism. Further, light intensity has been identified as a crucial factor affecting the structural genes and enzymatic activities involved in capsaicinoid synthesis (Arce-Rodríguez & Ochoa-Alejo, 2019). Optimal light intensity is essential for increasing the capsaicinoid content in chillies (Yang *et al.,* 2024).

The Scoville Heat Test, an organoleptic evaluation introduced by Scoville in 1912, has traditionally been used to determine pungency levels. However, the cumbersome organoleptic analysis has been replaced by directly measuring the capsacinoid content with HPLC (high-performance liquid chromatography) or GC (gas chromatography) (Duelund and Mouritsen, 2017). High-Performance Liquid Chromatography (HPLC) is considered as the most reliable and rapid technique for the estimation of capsaicinoids (Collins *et al.*, 1995; Stoica *et al.*, 2016).

The present study aims to quantify the capsaicin content and assess the pungency variations among seven accessions of Dalle Khursani collected from the Darjeeling-Sikkim Himalayas by employing HPLC for precise capsaicinoid estimation. The findings are expected to provide a foundation for the conservation, improvement, and commercial exploration of this valuable landrace.

Table 1. List of Dalle Khursani accessions and their place of collection

Sl. No.	Accession	Place of collection	Altitude
1	DK-01	Sukhiapokhri, Darjeeling	2194 m
2	DK-02	Bijanbari, Darjeeling	752 m
3	DK-03	Mirik, Darjeeling	1495 m
4	DK-04	Bongbusty, Kalimpong	1003m
5	DK-05	Gitdabling, Kalimpong	1156 m
6	DK-06	Pedong, Kalimpong	1356 m
7	DK-07	Kafer, Kalimpong	1523 m

Materials and methods

Fruits of Dalle Khursani were collected from seven different altitudinal locations at the fully ripened stage (Table. 1). As this typical landrace is generally consumed fresh with seeds, the entire fruit along with seeds and placenta was considered for determining the capsaicin content.

The entire work was carried out at the Central Instrument Centre and Quality Control Laboratory, Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal, India.

Standard capsaicin (12084-10G) was obtained from Sigma Aldrich Co. (USA) and used as external standards. HPLC-grade methanol and acetonitrile were used. HPLC-grade water was used throughout the experiment. An HPLC system (Waters Corp., Milford, MA) with a single 515 solvent delivery pump and a 2489 UV/Vis Detector was used for high-throughput isocratic analyses. The Novapak C18 column (150 mm × 4.6 mm, 5 µm) was maintained at 30 ^{o}C. The mobile phase was isocratic with 70% solvent B (100% methanol) and 30% solvent A (10% methanol solution v/v) with a flow rate of 1 mL/min for 10 minutes. The mobile phase was run through the system for 45 minutes to equilibrate the column before injecting 20µL of the sample. The UV detection wavelength was set at 280 nm as it has the maximum absorbance of capsaicinoids.

The capsaicin standard was used to obtain a calibration curve based on the peak-area ratio for the known concentration of the external standard. A stock solution of the standard was prepared by dissolving 5 mg of capsaicin in 5 mL of acetonitrile and further diluted to desired concentrations in acetonitrile to generate the calibration curve.

The study included sample collection, where fruits were selected from seven altitudinal locations. These fruits underwent washing and drying before being processed into a fine powder through grinding (1 g of dried powder). The fruits without visible damage were selected, washed with double distilled water, and dried. Extractions and quantifications of capsaicin for all the accessions were done in triplicate using a modified HPLC procedure for the short-run method as described by Collins *et al.* (1995). Acetonitrile was used to extract 1g of dried chilli powder for each sample in 10 mL of solvent as was done by Stewart *et al.* (2005). The samples were placed in a water bath at 80 ^{o}C for four hours, with occasional swirling at hourly intervals, and then allowed to cool at room temperature. About 2 mL of supernatant from each sample was extracted and filtered through a polyethylene membrane of 0.45 µm of pore size and stored at 5 °C in a refrigerator until analysis.

The extracted samples were subjected to HPLC analysis using a Waters 2489 HPLC system equipped with a Novapak C18 column. The flow rate was set at 1 mL/min for 10 minutes, and the detection wavelength was 280 nm. A calibration curve was prepared by dissolving standard capsaicin in acetonitrile to generate a relationship between peak area and concentration.

For capsaicin quantification, 20 µL of each sample was injected into the HPLC system and analyzed in triplicate for the seven accessions. The data obtained were subjected to data analysis, where capsaicin content was converted to mg/g of dry weight and expressed in Scoville Heat Units (SHU).

The determination of capsaicin content in the sample extracts was performed using external standards. The standard solutions were run on the HPLC and the standard curves were generated by plotting peak area against concentration. Capsaicin content was converted into Scoville Heat Units (SHU) based on concentration values (Helrich, 1998). The chromatogram presented in this study was generated using Empower 3 HPLC software, which facilitated the accurate analysis and visualization of the data.

Statistical analysis: Three replicates were performed for each of the seven accessions. Capsaicin concentration was expressed in mg/g of dried weight. ANOVA and LSD tests were used for the statistical analysis of the results and analysed using Grapes version 1.1.0 software (Gopinath *et al.,* 2021).

Results and discussion

The study revealed significant variation in capsaicin content among the seven Dalle Khursani accessions, ranging from 19.05 mg/g to 28.54 mg/g, corresponding to Scoville Heat Units (SHUs) of 304,863 to 456,636 (Table. 2). DK-03 exhibited the highest capsaicin concentration (28.54 mg/g), closely followed by DK-02 (28.42 mg/g). Interestingly, these two accessions did not show significant differences in capsaicin levels despite growing in vastly different environments, suggesting a strong genetic influence. However, they differed significantly from the other accessions, underscoring the presence of considerable genetic variability for pungency within the Dalle Khursani population.

Environmental factors such as microclimate, soil composition, and water availability likely played a secondary role in influencing capsaicin levels, as reflected in the variability observed among accessions from similar altitudes. For instance, DK-03 and DK-02 outperformed accessions from both higher altitudes, like DK-01, and mid-altitudes, such as DK-06 and DK-07. The variations in capsaicin content (mg/g) across altitudes in Dalle Khursani accessions are depicted in Fig. 1. The capsaicin content did not appear to be correlated with the altitude of the location which corroborates with the earlier findings of Thapa *et al.* (2009). These findings suggest that capsaicin synthesis is predominantly governed by genetic factors rather than altitude-specific environmental conditions. This observation underscores the complex interplay of genetics and micro-environmental factors in shaping the pungency levels of chilli landraces.

Accessions DK-03 and DK-02 could be valuable resources for breeding programs aimed at developing high-pungency varieties, while mid-altitude accessions like DK-06 and DK-05 could be targeted for stable capsaicin production under similar agro-climatic conditions. This study offers valuable knowledge for enhancing the cultivation practices and commercial potential of Dalle Khursani.

Table 2. Capsaicin content and pungency level in seven accessions of Dalle Khursani

Accession	Capsaicin (mg/g)	Scoville Heat Units (SHU)	Pungency level
DK-01	23.127±1.171c	370,015±18729.33c	VHP
DK-02	28.423±0.581a	454,805±9295.98a	VHP
DK-03	28.540±0.651a	456,636±10466.25a	VHP
DK-04	19.050±0.340d	304,863±5476.68d	VHP
DK-05	26.543±0.435b	424,741±6984.51d	VHP
DK-06	26.607±0.190b	425,721±3080.52b	VHP
DK-07	20.053±0.946d	320,869±15145.91b	VHP
Range	19.050-28.540	304,863-456,636	
Mean	24.620	393,950	
SEm (±)	0.430	6889.957	
CD (0.05)	1.325	21230.071	
CV	3.026	3.029	

Each value is an average of three samples (±) standard error of the mean. The means followed by different letters in the same column are significantly different (P=0.05) by the LSD test. VHP: Highly pungent

The capsaicin contents obtained from different fruits were converted to SHU (Table. 2) to classify them according to their various pungency levels as per the method given by Helrich (1998). All the accessions of Dalle Khursani analysed in the present study can be classified as very highly pungent as the Scoville Heat Units (SHU) values exceed 80,000 (Weiss, 2002). A representative chromatogram depicting the distinct peak of capsaicin from a Dalle Khursani accession (DK-03 collected from Mirik, Darjeeling) has been presented in Fig. 2.

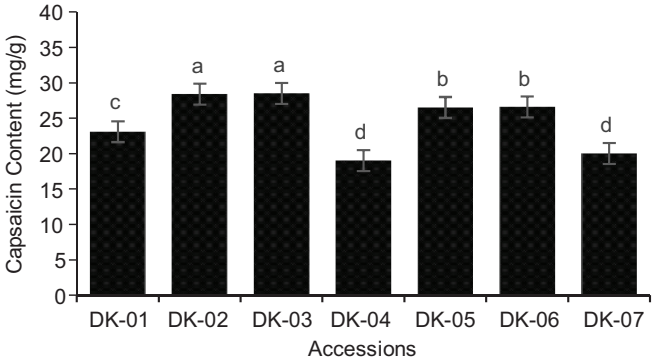

Fig. 1. Variation in capsaicin content (mg/g) in Dalle Khursani accessions

Available literature suggests that *Capsicum chinense* species includes the most pungent chilli peppers (Bosland and Baral, 2007; Canto-Flick *et al.,* 2008) and some authors have also mentioned Dalle Khursani as *C. chinense*. But recent works by Jha *et al.* (2017) indicated its closer affinity to *Capsicum annuum*, and have reported the polyploid nature of this landrace.

Information on the pungency level of Dalle Khursani is scanty and reported to be in the range of 100,000 to 350,000 SHU (Lepcha *et al.,* 2023) similar to that of Habanero pepper. Our study shows that the SHU values for Dalle Khursani can exceed 450,000, which is higher than previously documented levels, suggesting a much broader variability than earlier believed. The capsaicin content and corresponding pungency level of these accessions can be expected to have a higher level if only the placenta has been used for the analysis as capasaicin is produced in placental tissues.

Dalle Khursani, a Geographical Indication (GI)-tagged chilli, exemplifies the unique characteristics tied to its specific growing region, making it an asset in Capsicum research. It emphasises the critical role of local landraces in preserving genetic diversity, serves as a model for studying genotype-environment interactions that influence traits such as high capsaicin content, and distinct flavour profiles, and expands the genetic resources available for breeding programs aimed at improving stress tolerance, yield, and quality in *Capsicum* species.

The pungency in peppers varies with environment, genotype or cultivar, node position, fruiting and maturity stages, and nitrogen and potassium contents (Uarrota *et al.*, 2020). When compared to other hot pepper landraces globally, such as Bhut Jolokia from India or Habanero from Mexico, Dalle Khursani stands out for its distinctive spherical shape, vibrant red colour, and intense pungency, rivalling some of the hottest varieties globally. Its exceptional adaptability to the Himalayan environment provides valuable information for breeding chilli suited to marginal and stress-prone conditions.

There is huge literature available on capsaicin content in other cultivated Capsicum species like *C. chinense*, *C. frutescens*, and *C. annuum* (Bosland and Baral, 2007; Canto-Flick *et al.*, 2008). Although Dalle Khursani has a recognised GI status, no systematic analysis of capsaicin variation across accessions from different

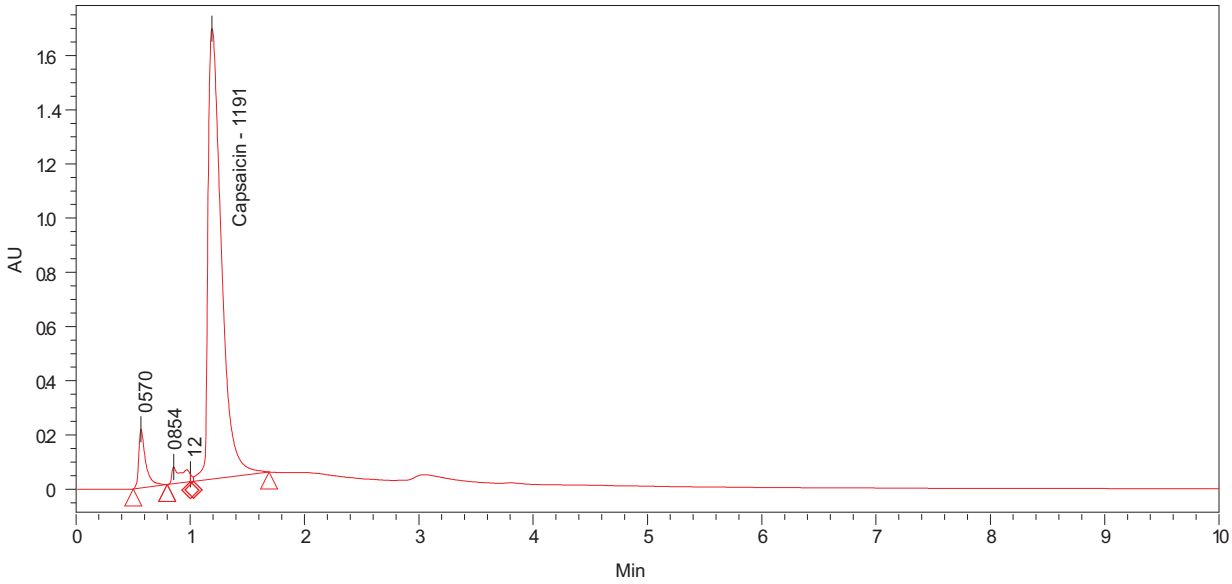

Fig. 2. Chromatogram of capsaicin content in Dalle Khursani accession (DK-03)

altitudinal zones has been reported. This study successfully addressed critical gaps in existing research by offering the first quantitative evaluation of capsaicin content and pungency levels across seven distinct accessions of Dalle Khursani collected from different altitudes in the Darjeeling-Sikkim Himalaya region. The variation in capsaicin content among the cultivars could be attributed to genotype differences (Tirumalachar, 1967) or may be due to the presence of gene modifying factors for pungency and the ratio of placental tissue to seed and pericarp (Sreelathakumary, 2000). Sampling from diverse elevations, as in this study, likely contributed to the observed variation, aligning with findings by Islam *et al.* (2015) in Bhut Jolokia accessions.

The most comprehensive earlier study on Dalle Khursani by Gurung *et al*. (2011) involved 14 chilli cultivars grown at four altitudes in Bhutan and Thailand. Significant variations in capsacinoid concentrations were influenced by cultivar and elevation, with soil conditions having minimal impact. Surprisingly, a negative correlation between temperature, sunlight, and capsaicinoid content was noted, likely due to increased vegetative growth at higher temperatures at the expense of capsaicinoid formation.

This study provides essential baseline data for future breeding programs focused on stabilising yield and pungency traits. It also positions Dalle Khursani as a premium spice product with market potential for value-added applications in functional foods and nutraceuticals. Conservation strategies, including sustainable cultivation practices, are critical to preserving this unique landrace and ensuring its long-term productivity in the face of climate change.

Despite its prominence for its pungency, Dalle Khursani remains underexplored in terms of genetic characterisation. Comprehensive genetic studies, such as genome-wide association studies (GWAS) and high-throughput sequencing, are essential to unravel its genetic diversity and identify key genomic regions associated with desirable traits. A deeper understanding of its polyploid nature can provide clarity into the genetic redundancy and variability underlying its phenotypic traits, including pungency, stress tolerance, and yield. Such genetic profiling can also aid in conserving this unique germplasm and incorporating it into breeding programs for trait improvement.

The postharvest handling practices significantly influence capsaicin stability and overall pungency in chilli peppers. For Dalle Khursani, optimising postharvest practices is crucial to maintaining its distinctive pungency. Studies focusing on low-temperature drying, controlled atmosphere storage, and the use of antioxidant treatments could help in preserving capsaicin levels.

This study provides essential baseline data to support future breeding programs focused on stabilising yield and pungency traits. It highlights accessions such as DK-02 and DK-03 as superior in capsaicin content, making them potential candidates for further selection and genetic improvement. The high capsaicin levels observed in this study establish Dalle Khursani as a premium spice product, comparable to globally recognised pungent varieties such as Habanero and Bhut Jolokia. This creates opportunities for enhanced market linkages and value-added products, offering significant benefits to growers in the Darjeeling-Sikkim region. Conservation strategies need to be prioritised to safeguard this unique landrace, especially in the face of changing climate conditions, by encouraging sustainable cultivation practices that will not only enhance productivity but also support the long-term conservation of this GI-tagged crop.

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