CHAPTER IV
Nutritional and Morphological Characterization of New Mungbean (*Vigna radiata* l.) Lines: Implications for Sprout Quality and Environmental Variation

4.1 Abstract

This study aimed to characterize the nutritional composition of seeds and sprouts derived from newly developed mungbean lines, and to assess their morphological characteristics as well as the influence of environmental variation on nutritional quality. The proximate nutritional composition of mungbean (*Vigna radiata* (L.) R. Wilczek) seeds and sprouts, including moisture, crude protein, crude fat, total ash, crude fiber, and carbohydrates, along with the morphological traits of sprouts (hypocotyl diameter, hypocotyl length, root length and output ratio) were evaluated across seven genotypes comprising two check varieties (CN3 and CN84-1) and five newly developed lines (P08, P12, P22, P24, and D5). Plants were cultivated under two contrasting environments: Phitsanulok during the rainy season (PNR) and Chai Nat during the dry season (CND). Significant effects of genotype (G), environment (E), and genotype-by-environment interaction (GEI) were detected for most nutritional traits, highlighting the complexity of nutritional variation. Seeds and sprouts of plants grown under PNR conditions exhibited higher fat and carbohydrate contents, whereas those from CND had elevated protein and ash levels. The check varieties CN3 and CN84-1 consistently showed high protein content, while lines P08 and P24 were superior in carbohydrate accumulation in both seeds and sprouts. Line P12 demonstrated high fiber content in seeds, whereas P22 was notable for fiber enrichment in sprouts. Moreover, P24 exhibited elevated ash content in sprouts. Crude fat levels in both seeds and sprouts showed only minor variation across genotypes and environments. Morphologically, root length was the only trait that discriminates among genotypes. Line D5 exhibited desirable sprout characteristics, including short roots and high output ratio, beneficial for commercial production. These findings confirm the potential of the new mungbean lines, with nutritional and morphological performance. Furthermore, environmental conditions significantly influenced nutrient accumulation, suggesting their importance in breeding programs targeting seed quality improvement.

4.2 Introduction

Mungbean (*Vigna radiata* (L.) R. Wilczek) is a legume crop of global importance, especially in tropical regions, where it is widely cultivated due to its nitrogen-fixing ability by symbiosis with *Rhizobium* sp. (Chaiyapan et al., 2023). This characteristic makes it a valuable soil-enriching crop, commonly used in crop rotations or as green manure to improve soil fertility. The crop is economically and nutritionally significant, serving as an essential source of food and feed. Mungbean seeds are also a rich source of macronutrients, comprising up to 67.10% carbohydrates and 32.60% protein content (Dahiya et al., 2015). Their high protein content not only contributes to their nutritional value but also makes mungbean an excellent candidate for hydrolysate production (Karami and Duangmal, 2024). Moreover, their compositional profile supports their versatility as raw material for a wide range of processed food products (Bhatty et al., 2000). Mungbean sprouts are highly regarded for their enhanced digestibility and enriched profile of phytonutrients, including vitamins C, A, B1, B2, and gamma-aminobutyric acid (GABA), which contribute to their functional food value (Randhir and Shetty, 2005). Both mungbean and black gram (*Vigna mungo*) are widely utilized in sprout productions due to their rapid germination and favorable nutritional profiles (Masari et al., 2016). Beyond their nutrient composition, mungbean consumption has been linked to a range of health-promoting effects, including antioxidant, anticancer, and anti-inflammatory activities (Hou et al., 2019). Furthermore, mungbean-derived products such as vermicelli, characterized by a low glycemic index (GI), have shown potential in supporting glycemic control and cardiovascular health, particularly in individuals with diabetes and related metabolic disorders (Yeap et al., 2012; Hou et al., 2019).

The nutrient composition of plants is governed by a complex interplay of genetic factors, environmental conditions, and their interactions (genotype-by-environment interactions; GEI), all of which critically influence both the quantity and quality of nutrients accumulated in seeds (Pregitzer et al., 2013; Cong et al., 2015; Asaro et al., 2016; Napier et al., 2023). Understanding these relationships is essential for advancing breeding strategies and agronomic practices aimed at improving the nutritional value of legumes. Nutrient accumulation is a dynamic process, particularly intensified during the reproductive stage, and individual nutritional traits often respond differently to genetic and environmental influences. For example, in maize, crude protein content is predominantly affected by environmental conditions, while lipid accumulation is more strongly determined by genetic factors (Cong et al., 2015). GEI is also a key contributor to phenotypic variation in agronomic and nutritional traits

(Napier et al., 2023). In legumes, traits such as protein concentration and anti-nutritional factors like phytate are significantly influenced by genotype, environment, and their interactions (Gore et al., 2021). Environmental variables, including light parameters, also play an important role in legume growth and nutrient accumulation (Vaidya and Stinchcombe, 2020). Additionally, factors such as soil properties and moisture levels impact the symbiotic relationship between legumes and *Rhizobium* spp., which is vital for nitrogen fixation and thus seed nutrient content (Yeremko et al., 2025).

Recently, several new mungbean lines with enhanced resistance to major fungal diseases powdery mildew (PM) caused by *Sphaerotheca phaseoli* and Cercospora leaf spot (CLS) caused by *Cercospora canescens* and high yield potential have been developed, including line D5 (Papan et al., 2024) and lines P08, P12, P22, and P24 (Pookhamsak et al., unpublished data). While these lines represent promising genetic resources for sustainable mungbean production, their nutritional profiles, particularly concerning seasonal or environmental variation, have not yet been investigated. In contrast, the nutritional composition of existing commercial mungbean varieties has been relatively well documented. For instance, variety CN3 contains approximately 58.37% carbohydrate, 24.05% protein, 1.03% fat, 4.50% fiber, and 4.12% ash, while CN36 and CN72 have reported carbohydrate contents of 56.17% and 56.35%, respectively (Jomsangawong et al., 2022). Moreover, Department of Agriculture (2018) also reported that the variety CN84-1 contained 54.85% carbohydrate. Overall, mungbean seeds typically contain 50.00–70.00% carbohydrates and 20.00-30.00% protein, although these values can vary depending on genotypes and environmental conditions (Somta et al., 2022). Despite this existing knowledge, it remains unclear whether the improved disease-resistant lines also possess favorable nutritional characteristics comparable to or exceeding those of established varieties.

The present study aimed to characterize the nutritional compositions of seeds and sprouts derived from these newly developed mungbean lines and to assess the influence of environmental variation on their nutritional quality. The results may offer valuable insights for the food industry in selecting mungbean lines with superior nutritional attributes and provide a foundation for breeding programs targeting enhanced nutritional value in mungbean.

4.3 Materials and Methods

4.3.1 Plant materials

Seeds of seven mungbean genotypes (CN3, CN84-1, P08, P12, P22, P24, and D5) were collected from the regional yield trials conducted at two locations in Thailand during the 2023–2024 growing seasons: the Phitsanulok Seed Research and Development Center (Phitsanulok province) during the rainy season (PNR), and the Chai Nat Field Crops Research Center (Chai Nat province) during the dry season (CND). Standard agronomic practices were followed during seed sowing and crop management to ensure uniform growth and development across genotypes. Plants were grown to physiological maturity, defined as 70 days after planting (DAP), at which point more than 80% of the pods had reached maturity. The experimental design consisted of plots measuring 4 × 6 m per replication, arranged in a randomized complete block design (RCBD) with three replications. Each plot contained 8 rows with a row spacing of 0.5 m and intra-row plant spacing of 0.2 m, totaling 30 plants per row. Supplemental irrigation was provided weekly via sprinkler systems to maintain optimal soil moisture. The soil characteristics and environmental conditions of these sites were presented in Table A.1 and Figure A.8 and Figure A.11, respectively. Additionally, details of the mungbean genotypes along with their specific characteristics were provided in Table 4.1.

Table 4.1 The information and characteristics of seven mungbean genotypes.

Genotypes	Pedigree	Special features	Descriptions
CN3	Selection from mutated CN36	Large seed, high yield, uniform maturity	Thai certified varieties developed at Chai Nat Field Crops Research Center, Thailand
CN84-1	Selection from mutated CN36	Large seed, high yield, high percentage of carbohydrate	
P08	Selected from backcrossing between CN84-1 and double cross lines [(CN72×V4758) × (CN72×V4718)] × [(CN72×V4718) × (CN72×V4785)] (V4718, V4758, and V4785 were originated from India) (Pookhamsak et al., unpublished data)	Large seed, uniform maturity, moderate resistance to PM[1/] and CLS[2/]	New resistant lines
P12		High yield, rather drought resistance, high resistance to PM, moderate resistance to CLS	
P22		High yield, uniform maturity, abundant pods, moderate resistance to PM and CLS	
P24		Large seed, uniform maturity, moderate resistance to PM and CLS	
D5	Selected from backcrossing between SUT1 and double cross lines [(CN72×V4758) × (CN72×V4718)] × [(CN72×V4718) × (CN72×V4785)] (SUT1 Variety developed at SUT, Thailand) (Papan et al., 2024)	Uniform maturity, moderate resistance to PM, abundant pods, pods borne above the canopy, and trichomeless	

[1/] powdery mildew, [2/] Cercospora leaf spot

4.3.2 Samples preparation

Seed samples were prepared by removing impurities, drying them at 45°C for 24 hrs, and then grinding them into a fine powder. The powdered samples were subsequently stored at -20°C to preserve their integrity for nutritional analysis. Sprout samples were prepared using a modified method based on Wang et al. (2021). Mungbean seeds were washed with sterile distilled water and soaked in warm water at 40°C for 30 min. After soaking, the seeds were kept in dark conditions at room temperature for 8 hrs. Following the soaking period, the seeds were rinsed with sterile distilled water and germinated using the between paper (BP) method. The germination process was conducted in a dark, temperature - controlled chamber at 25°C, with the seeds watered twice daily using sterile distilled water for 72 hrs. After germination, the sprouts were dried at 45°C for 24 hrs and ground into a fine powder. The powdered samples were then stored at -20°C for subsequent nutritional analysis.

4.3.3 Proximate analysis

The contents of crude protein, crude fat, crude fiber, and total ash were analyzed following the procedures outlined by the Association of Official Analytical Chemists (AOAC, 2019). The total carbohydrate content was calculated by deducting the sum of these proximate components from the total. The parameter was measured in triplicates.

4.3.3.1 Moisture content

The measurement of moisture content, petri dish was placed in a hot air oven at 105°C for 2 hrs. Then, it was placed in a desiccator for 30 min until the temperature of the petri dishes equaled room temperature. Next, the petri dish was weighed using an analytical balance to four decimal places, 2-3 g of sample was placed into the petri dish with the cover slightly ajar and returned to the hot air oven at 105°C for 2 hrs or until dried. After drying, the petri dish containing the sample was allowed to cool in the desiccator for 30 min until the temperature of the petri dishes equaled room temperature. Then, the petri dish with the dried sample was weighed using the analytical balance to four decimal places. The drying process at 105°C was repeated for 1 hr or until the weight difference between two consecutive weighing was no different than 3 mg. The moisture content was calculated using the following equation:

$$\text{Moisture Content (\%FW)} = \frac{(\text{W2} - \text{W3})}{(\text{W2} - \text{W1})} \text{x } 100$$

When: W1 = Weight of the empty petri dish (g)
W2 = Weight of the petri dish with sample before drying (g)
W3 = Weight of the petri dish with the dried sample (g)

4.3.3.2 Crude protein content

The total protein content was determined using the Kjeldahl method. Approximately 0.5-1.0 g of sample was placed into a digestion tube, followed by the addition of 3 g of accelerating agent (a mixture of copper sulfate and potassium sulfate in a 1:10 ratio) and 20 mL of concentrated H_2SO_4. A blank sample was prepared by omitting the sample. Digestion was carried out in a Digestion System at 380 °C for 100 min until a clear blue solution was obtained. After digestion, the tubes were removed and cooled for about 20 min before proceeding to protein distillation using a UDK 149 Automatic Kjeldahl Distillation Unit (VELP Scientifica, Italy). For distillation, 20 mL of 4% boric acid (H_3BO_3) solution mixed with 2–3 drops of mixed indicator (methyl red and bromocresol green at a 1:1 ratio) was prepared in an Erlenmeyer flask for each sample. The distillation was performed with the following program: 45 mL H_2O, 60 mL of 40% NaOH, and a distillation time of 4 min. During distillation, ammonia gas (NH_3) released from the sample reacted with NaOH and condensed into the boric acid solution, causing a color change from pink to green. The resulting solution was then titrated with 0.1 N HCl until the endpoint, indicated by a color change back to pink, was reached. The volume of HCl used was recorded.

$$\text{Crude protein content (\%DB)} = \frac{(\text{A} - \text{B}) \text{ x N x } 1.4007 \text{ x F}}{\text{W}_\text{t}}$$

When: A = Volume of HCl used for sample titration (ml).
B = Volume of HCl used for blank titration (ml).
W_t = Weight of the sample (g).
N = Concentration of the HCl (N).
F = Factor (specific to mungbean, which is 6.25).

4.3.3.3 Crude fiber content

Crude fiber analysis was performed using the Fibertec 2010 automatic analyzer (Foss Tecator, Denmark). Approximately 0.5-1.0 g of sample was placed into a filtered crucible. Then, 150 mL of 1.25% hot H_2SO_4 was added to each tube, along with 3 drops of n-octanol antifoaming agent to minimize foaming. The sample was boiled for 45 min. After boiling, the mixture was filtered until dry by activating air

suction, followed by releasing the acid from the sample through valve opening. The samples were washed three times with hot H_2O and filtered to dryness. Next, 150 mL of 1.25% NaOH solution was added, again with 3 drops of n-octanol antifoam, and the sample was boiled for 45 min. After boiling, the sample was filtered and washed three times with hot H_2O. Subsequently, the sample was rinsed with acetone (C_3H_6O) 3 times, each with 25 mL. The filtered crucible containing the residue was removed from the extractor and dried in an oven at 105 ℃ for 2 hrs, then cooled in a desiccator for 30 min. Following drying, the crucible was incinerated in a muffle furnace at 500 ℃ for 2 hrs. The furnace was allowed to cool until the temperature fell below 250 ℃ before opening; the furnace must remain closed for at least 3 hrs prior to sample removal or until the temperature is below 200 ℃. Finally, the crucible was cooled again in a desiccator for 30 min before further analysis.

$$\text{Crude fiber content (\%DB)} = \frac{W_2 - W_3}{W_1} \text{ x } 100$$

When: W_1 = Weight of sample (g).
W_2 = Weight of sample + crucible after oven dry (g).
W_3 = Weight of sample + crucible after burn in furnace (g).

4.3.3.4 Ash content

Analysis of ash content started with weighing the constant weight in hot air oven at 105℃ for 2 hrs, 2-3 g of the dried sample put into a crucible. The samples were incinerated on a hot plate until smokeless to form a lump. Subsequently, incinerate in a furnace at 500℃ for 3 hrs or until a light gray or uniform white ash is obtained. After removal from the furnace, it is allowed to cool to room temperature in a desiccator for 30 min. The weight was recorded, and the ash content calculated according to the following formula:

$$\text{Ash content (\%DB)} = \frac{W_2 - W_1}{S} \text{ x } 100$$

When: W_1 = Weight of the crucible.
W_2 = Weight of the crucible and sample after incineration.
S = Weight of the sample.

4.3.3.5 Fat content

The analysis of fat content was conducted following the Soxhlet extraction method using the Soxtec™ 2050 Auto Fat Extraction System. Initially, the extraction beaker was weighed and dried at 105°C for 1 hr to achieve a constant weight, then cooled in a desiccator. Approximately 1–1.5 g of the sample was weighed and put into the filter paper, then folded and inserted into a cellulose thimble. The cellulose thimble was positioned for extraction and insertion of the extract. Subsequently, 80 mL of petroleum was added to the extraction beaker and put it into the positioned for extraction. The heating program was set with the following parameters: extraction temperature at 180°C, extraction phase: 60 min, rinsing phase of 90 min, and drying phase for 15 min. Upon completion of the program, the extraction beaker containing the extracted fat was removed and dried in a hot air oven at 105°C for 2 hrs. After drying, the beaker was cooled to room temperature in a desiccator for 30 min. Finally, the extraction beaker with the extracted fat was weighed to determine the fat content.

$$\text{Crude fat content (\%DB)} = \frac{\text{B} - \text{A}}{\text{W}} \text{ x } 100$$

When: W = Weight of the sample.
A = Constant weight of extraction beaker.
B = Weight of extraction beaker and extracted fat.

4.3.3.6 Carbohydrate Content

To analyze the carbohydrate content, using the following method outlined by Hailu (2018). Carbohydrate content calculated by subtracting the percentages of moisture, protein, fat, fiber, and ash according to the following formula:

$$\text{Carbohydrate content (\%DB)} = 100 - \text{Moisture} + \text{ Protein } + \text{Fiber } + \text{Fat } + \text{Ash}$$

4.3.4 Morphological characteristics of mungbean sprouts

Data collection for hypocotyl diameter, hypocotyl length, and root length: Collected data from 20 sprouts/genotype/replication, with 6 replicates, and measured the results using a ruler and vernier caliper. Hypocotyl diameter (mm): Measure at the midpoint of the hypocotyl once per plant using a vernier caliper. Hypocotyl length (cm): Measure from the base of the hypocotyl to the cotyledon node using a ruler. Root length (cm): Measure from the base of the hypocotyl to the longest tip of the

root. Output ratio: Calculated based on data collected from 6 replicates for each genotype follow by modified method from (Wang et al., 2021), computed as output ratio = fresh weight of sprout (g) / weight of mungbean seeds (g)

A completely randomized design (CRD) was employed for the experimental setup. Data were analysed using the analysis of variance (ANOVA). A mean comparison was conducted using Duncan's New Multiple Range Test (DMRT) to assess the nutrient composition across mungbean genotypes and the morphological characteristics of mungbean sprouts. Additionally, a combined analysis was performed to investigate the effects of genotype, environment and interactions between genotype and the environment. Statistical analyses were conducted using SPSS version 16.0 (Levesque, 2007).

4.4 Results

The nutritional composition, including moisture, crude protein, crude fat, total ash, crude fiber, and carbohydrate contents, was evaluated in seeds and sprouts of seven mungbean genotypes. These included two certified check varieties (CN3 and CN84-1) and five newly developed lines (P08, P12, P22, P24, and D5). The genotypes were cultivated under two contrasting environmental conditions: Phitsanulok rainy season (PNR) and Chai Nat dry season (CND). Combined analysis of variance revealed that genotype (G), environment (E), and genotype-by-environment interaction (GEI) had significant effects ($P \leq 0.01$ or $P \leq 0.05$) on nearly all measured nutritional traits in both seeds and sprouts, though the magnitude and patterns of these effects varied among traits (Table 4.2 and Table 4.3). The significant GEI underscores the differential response of mungbean genotypes to varying environmental conditions. Overall, the nutritional composition patterns were consistent between seeds and sprouts, with CND-grown samples exhibiting significantly higher protein and ash contents, while those from PNR showed elevated fat and carbohydrate levels.

4.4.1 Proximate nutritional composition of mungbean seeds; genotypic, environmental, and genotype-by-environment interaction effects

The nutritional composition of mungbean seeds is summarized in Table 4.2. Moisture content was strongly influenced by G, E, and GEI. Seeds produced under the PNR environment exhibited significantly higher moisture content (11.19%) than those from the CND environment (8.63%), reflecting environmental differences in post-harvest humidity. The significant GEI effect was evident in the differential response of genotypes across environments. Under PNR, all new lines and CN84-1 (11.09-11.44%) had higher moisture content than CN3 (10.51%). Among them, P12 and P22 were comparable to CN84-1, while other new lines showed 1.01- to 1.03-fold lower values. In contrast, under CND, genotypic differences were more distinct: P12 exhibited the lowest moisture content (8.06%) and D5 the highest (9.36%). Notably, P12 and P22 showed significantly lower (1.03- to 1.08-fold) moisture content than both check varieties in CND. When averaged across environments, CN3 maintained the lowest overall moisture level (9.61%), while D5 exhibited the highest (10.28%), indicating combined G and GEI effects.

Crude protein content was significantly affected by G, E, and GEI. Seeds grown in the CND environment accumulated higher protein levels (25.64%) than those in PNR (24.01%). Across genotypes, CN3 and CN84-1 had the highest average protein contents (25.87% and 25.83%, respectively). All newly developed lines exhibited 1.03- to 1.09-fold lower protein contents than these checks. GEI effects were

apparent in shifts in genotype ranking between environments. For example, line D5 achieved 26.79% protein under CND, comparable to the checks, but displayed 1.07- to 1.08-fold lower content under PNR. Among new lines, P12 ranked highest in PNR (24.14%), while D5 performed best in CND, highlighting the genotype-specific responsiveness to environmental conditions.

As with crude protein content, crude fat content was significantly affected by all three sources of variation. But in contrast to protein, seeds grown under PNR conditions accumulated more fat (0.88%) than those grown under CND (0.54%). Significant GEI was evident, particularly in the divergent performance of genotypes across environments. Under PNR, P08 had the lowest fat content (0.49%), comparable to both check varieties. In CND, D5 recorded the lowest value (0.42%), and most new lines, except P24, exhibited 1.11- to 1.50-fold lower fat content than CN3 (0.63%). However, when averaged across both environments, P12 and P22 showed significantly higher crude fat contents (up to 1.53-fold) than the check varieties, while P08 remained comparable to CN3 and CN84-1, revealing a strong G effect modulated by E.

Total ash content was significantly influenced by E and GEI, though genotypic effects were not significant when averaged across environments. Under CND (overall means 4.67%), genotypic differences became evident. CN3 recorded the highest ash content (5.07%), while P24 had the lowest (4.26%). Nevertheless, all new lines, including P24, were statistically similar to CN84-1, indicating limited genotypic variation. Under PNR, differences among genotypes were less pronounced. These findings suggest that ash accumulation is more responsive to environmental variation and specific genotype-environment combinations than to genotype alone.

In contrast to most other nutritional traits, crude fiber content was not significantly influenced by E. However, both G and GEI had highly significant effects. Mean fiber content was similar between PNR (4.20%) and CND (4.40%). Line P12 demonstrated superior performance, with the highest average fiber content across environments (5.47%), significantly exceeding all other genotypes by 1.17- to 1.48-fold. Conversely, P08 had the lowest average fiber level (3.70%). When compared to the check varieties, lines P08 and D5 had similar crude fiber contents to CN3, whereas P22 and P24 were comparable to CN84-1. Fiber accumulation patterns varied across environments: under PNR, P12 again led (6.24%), while in CND, P24 had the highest value (4.84%). Notably, under CND, all new lines except P08 exhibited significantly higher fiber contents (4.19-4.84%) than the check varieties, with increases ranging from 1.01- to 1.19-fold, further illustrating GEI-driven variation.

Carbohydrate levels were significantly affected by G, E, and GEI. Seeds from the PNR environment had higher carbohydrate content (67.03%) than those from CND (64.74%). Lines P08 and P24 consistently exhibited the highest average carbohydrate contents across environments (67.46% and 67.09%, respectively), surpassing the check varieties CN3 and CN84-1 by 1.03- to 1.04-fold. Whereas the remaining new lines exhibited either higher or comparable values relative to the checks. Under PNR, P08 and P24 reached 1.02- to 1.06-fold higher carbohydrate levels than the check varieties, while lines D5 and P12 showed values comparable to CN84-1. In CND, P08 again led (65.97%), followed by P12, P22, and P24, all of which significantly exceeded the checks by 1.01- to 1.04-fold. These findings reflect both stable high-performing genotypes and environment-specific responses.

4.4.2 Proximate nutritional composition of mungbean sprouts; genotypic, environmental, and genotype-by-environment interaction effects

All proximate nutritional composition of mungbean sprouts was significantly affected by G, E, and GEI, as confirmed by a combined analysis of variance (Table 4). Each nutritional trait exhibited distinct patterns of variation influenced by these three factors. Environmental influence was particularly pronounced in moisture content. Sprouts cultivated under the PNR environment exhibited markedly lower moisture levels (81.58%) compared to those from CND (87.30%). Minimal genotypic variation was observed under CND. However, under PNR, genotypic differences became evident; check variety CN84-1 exhibited the highest moisture content (84.06%), significantly surpassing all new lines, which exhibited 1.03- to 1.05-fold lower moisture content. Across environments, CN84-1 and CN3 maintained the highest average moisture levels (85.37% and 85.07%, respectively), whereas P08 consistently showed lower moisture content, highlighting both G and GEI effects.

Crude protein levels were also significantly shaped by G, E, and GEI. Sprouts from CND-grown seeds contained higher protein content (31.51%) than those from PNR (27.97%), underscoring a strong environmental impact. CN84-1 had the highest average protein content across environments (31.00%), significantly outperforming all new lines by 1.02- to 1.11-fold. However, most of them showed comparable protein levels to CN3 except P24. Notably, GEI was evident: under PNR, CN84-1 again had the highest protein level (29.65%), significantly surpassing all other genotypes, followed by D5 and CN3. Whereas, in CND, protein contents among genotypes were more uniform, ranging from 29.83% to 32.35%. Lines P08, P12, and D5 were statistically similar to the check varieties, while P24 consistently exhibited the lowest protein content across both environments.

Crude fat content demonstrated strong G, E, and GEI effects. Under PNR, significant genotypic differences emerged, with lines P08, P24, and D5 showing substantially lower fat levels than CN3 (by 1.27- to 1.40-fold). In contrast, non-significant differences were detected under CND, where fat content ranged narrowly (0.86–1.02%). However, when averaged across environments, P12 and P22 stood out with significantly higher fat contents (1.38% and 1.42%, respectively), while CN3, CN84-1, P08, P24, and D5 had lower values, illustrating combined G and GEI effects.

Total ash content in mungbean sprouts was significantly influenced by all three factors. Under PNR, line P24 exhibited the highest ash content (5.25%), exceeding other genotypes by 1.16- to 1.47-fold. Most other new lines, excluding P08, were statistically similar to CN3 and CN84-1. Under CND, new lines (except P24) had higher ash contents (1.05- to 1.12-fold) than CN84-1, while P24 (4.34%) aligned with both checks. Across environments, P24 maintained the highest ash content (4.80%), outperforming the other genotypes by 1.07- to 1.15-fold, demonstrating strong genotypic superiority as well as GEI.

In contrast to the crude fiber in seeds, the fiber content in sprouts was significantly influenced by E, with sprouts under PNR showing significantly higher levels (3.99%) than those under CND (2.74%). Within the PNR environment, line P22 showed the greatest fiber accumulation, surpassing other genotypes by 1.37- to 4.59-fold, followed by P12. Under CND, fiber levels were more uniform, with P12, P22, P24, and D5 remaining comparable to CN3 and CN84-1. When averaged across both environments, P22 had the highest fiber content (4.65%), outperforming other genotypes by 1.21- to 2.45-fold, reflecting its genotypic potential.

Carbohydrate contents varied significantly with G, E, and GEI. The PNR environment promoted higher carbohydrate accumulation (62.44%) compared to CND (60.39%). Across environments, lines P08 and P24 recorded the highest carbohydrate levels (63.55% and 63.17%, respectively), outperforming others by 1.04- to 1.06-fold. Strong GEI effects were evident: under PNR, P08 achieved the highest content (67.09%), followed by P24, both exceeding other genotypes by 1.03- to 1.14-fold. Lines D5 and P12 remained comparable to the check varieties. In contrast, under CND, P24 led with 62.24%, significantly higher (1.02- to 1.04-fold) than most genotypes except P22, indicating its stable performance across environments. The remaining new mungbean lines had carbohydrate contents comparable to the check varieties CN3 and CN84-1.

Table 4.2 Proximate composition of seeds from seven mungbean genotypes (%DB[1/]).

Genotypes	Moisture			Crude protein			Crude fat			Total ash			Crude fiber			Carbohydrates		
	PNR	CND	Average	PNR	CND	Average	PNR	CND	Average	PNR	CND	Average	PNR	CND	Average	PNR	CND	Average
CN3	10.51 e[2/]	8.72 b	9.61 e	25.05 a	26.70 a	25.87 a	0.59 bc	0.63 a	0.61 bc	3.65	5.07 a	4.36	3.30 c	4.17 b	3.74 c	67.42 b	63.42 d	65.42 bc
CN84-1	11.44 a	8.51 c	9.97 b	25.22 a	26.44 a	25.83 a	0.66 bc	0.48 de	0.57 bc	4.27	4.68 abc	4.47	4.67 b	4.08 b	4.38 b	65.19 cd	64.32 c	64.75 c
P08	11.31 b	8.69 bc	10.00 b	23.72 bc	24.63 c	24.18 d	0.49 c	0.52 cd	0.50 c	3.58	4.72 ab	4.15	3.25 c	4.16 b	3.70 c	68.96 a	65.97 a	67.46 a
P12	11.40 a	8.06 e	9.73 d	24.14 b	24.76 bc	24.45 cd	1.13 a	0.57 bc	0.85 a	4.00	4.80 ab	4.40	6.24 a	4.69 a	5.47 a	64.50 d	65.18 b	64.84 c
P22	11.41 a	8.29 d	9.85 c	24.10 b	25.04 bc	24.57 c	1.19 a	0.55 bc	0.87 a	4.04	4.42 bc	4.22	4.69 b	4.68 a	4.68 b	65.98 c	65.31 b	65.65 b
P24	11.09 d	8.80 b	9.94 bc	22.40 d	25.14 b	23.77 e	0.86 ab	0.59 ab	0.73 ab	3.80	4.26 c	4.02	3.93 bc	4.84 a	4.39 b	69.01 a	65.17 b	67.09 a
D5	11.20 c	9.36 a	10.28 a	23.41 c	26.79 a	25.1 b	1.22 a	0.42 e	0.82 a	3.90	4.75 ab	4.32	3.32 c	4.19 a	3.76 c	68.15 ab	63.84 cd	65.99 b
Mean	11.19	8.63	9.91	24.01	25.64	24.82	0.88	0.54	0.71	3.89	4.67	4.28	4.20	4.40	4.30	67.03	64.74	65.89
G[3/]	**	**	**	**	**	**	**	**	**	ns	*	ns	**	**	**	**	**	**
E			**			**			**			**			ns			**
G x E			**			**			**			*			**			**
C.V. (%)			0.90			1.19			18.98			6.27			9.70			0.88

[1/] DB = dry basis, [2/] Means in the same column with different letters are significantly different based on Duncan's New Multiple Range Test (DMRT), and [3/] * = Significant at $P \leq 0.05$; ** = highly significant at $P \leq 0.01$, and ns=non-significant at $P > 0.05$. Abbreviation: PNR= Phitsanulok (rainy season), CND = Chai Nat (dry season), G= Genotype, and E = Environment.

Table 4.3 Proximate composition of sprouts from seven mungbean genotypes (%DB[1/]).

Genotypes	Moisture			Crude protein			Crude fat			Total ash			Crude fiber			Carbohydrates		
	PNR	CND	Average	PNR	CND	Average	PNR	CND	Average	PNR	CND	Average	PNR	CND	Average	PNR	CND	Average
CN3	82.20 ab[2/]	87.93	85.07 a	28.48 b	31.70 ab	30.09 bc	1.43 b	0.86	1.15 b	4.12 bc	4.53 bcd	4.33 b	4.37 c	2.98 a	3.68 bc	61.59 cd	59.92 b	60.76 bc
CN84-1	84.06 a	86.68	85.37 a	29.65 a	32.35 a	31.00 a	1.07 c	0.97	1.02 c	4.50 b	4.26 e	4.38 b	3.05 e	2.94 a	3.00 d	61.73 cd	59.48 b	60.60 bc
P08	79.83 c	86.76	83.29 b	26.86 d	32.05 a	29.45 c	1.02 c	0.86	0.94 c	3.57 c	4.76 a	4.17 b	1.46 f	2.33 b	1.90 e	67.09 a	60.00 b	63.55 a
P12	80.82 bc	88.39	84.60 ab	27.70 c	31.23 ab	29.46 c	1.84 a	0.91	1.38 a	4.17 bc	4.74 ab	4.45 b	4.98 b	2.72 ab	3.85 b	61.31 d	60.40 b	60.86 bc
P22	81.27 bc	87.86	84.57 ab	28.42 b	30.75 bc	29.59 c	1.82 a	1.01	1.42 a	4.09 bc	4.60 abc	4.35 b	6.71 a	2.60 ab	4.65 a	58.96 e	61.04 ab	60.00 c
P24	81.53 bc	86.57	84.05 ab	26.13 e	29.83 c	27.98 d	1.03 c	1.02	1.02 c	5.25 a	4.34 de	4.80 a	3.51 de	2.57 ab	3.04 d	64.09 b	62.24 a	63.17 a
D5	81.34 bc	86.92	84.13 ab	28.57 b	31.94 ab	30.25 b	1.13 c	0.87	1.00 c	4.12 bc	4.49 cd	4.31 b	3.87 d	3.02 a	3.44 c	62.31 c	59.69 b	61.00 b
Mean	81.58	87.30	84.43	27.97	31.41	29.68	1.33	0.93	1.31	4.26	4.53	4.39	3.99	2.74	3.36	62.44	60.39	61.41
G[3/]	*	ns	*	**	**	**	**	ns	**	**	**	**	**	**	**	**	*	**
E			**			**			**			**			**			**
G x E			**			**			**			**			**			**
C.V. (%)			1.23			1.76			8.39			5.48			7.75			1.16

[1/] DB = dry basis, [2/] Means in the same column with different letters are significantly different based on Duncan's New Multiple Range Test (DMRT), and [3/] * = Significant at P ≤ 0.05; ** = highly significant at P ≤ 0.01, and ns=non-significant at P > 0.05. Abbreviation: PNR= Phitsanulok (rainy season), CND = Chai Nat (dry season), G= Genotype, and E = Environment.

4.4.3 Morphological traits of mungbean sprouts

The experimental results on the morphological characteristics of seven mungbean genotypes are summarized in Table 4.4. A significant difference was observed in root length, while hypocotyl diameter, hypocotyl length, and output ratio of mungbean sprouts showed non-statistically significant differences. The average hypocotyl diameter was 2.23 mm, with similar values observed across genotypes. Line P22 exhibited the largest diameter (2.31 mm), while D5 had the smallest (2.16 mm). In terms of hypocotyl length, CN84-1 recorded the longest hypocotyl (5.41 cm), followed by P24 and D5 (4.80 cm). The shortest hypocotyl was observed in line P12 (3.88 cm). Root length showed significant variation among genotypes. Genotypes CN84-1 and P24 produced longer roots (6.64 and 6.60 cm, respectively), 1.24- to 1.27-fold and 1.23- to 1.26-fold significantly higher than CN3 and D5, respectively. Lines P08, P12, and P22 had similar root lengths, with non-significant differences compared to each other and other genotypes. Non-statistically significant difference observed in the output ratio among the genotypes. The average output ratio was 4.27, which CN84-1 and D5 tending to produce higher ratios (4.58 and 4.52, respectively), while variety CN3 (3.77) tended to have a lower output ratio than the other genotypes.

Table 4.4 Morphological characteristics of seven mungbean genotypes sprouts.

Genotypes	Hypocotyl diameter (mm)	Hypocotyl length (cm)	Root length (cm)	Output ratio
CN3	2.24 ± 0.05	4.06 ± 0.36	5.23 ± 0.39 b	3.77 ± 0.10
CN84-1	2.24 ± 0.05	5.41 ± 0.47	6.64 ± 0.35 a	4.58 ± 0.29
P08	2.27 ± 0.06	4.64 ± 0.41	6.20 ± 0.20 ab	4.36 ± 0.48
P12	2.23 ± 0.09	3.88 ± 0.26	5.75 ± 0.32 ab	4.09 ± 0.18
P22	2.31 ± 0.06	4.33 ± 0.32	5.95 ± 0.44 ab	4.25 ± 0.26
P24	2.21 ± 0.04	4.80 ± 0.45	6.60 ± 0.24 a	4.34 ± 0.27
D5	2.16 ± 0.06	4.80 ± 0.48	5.37 ± 0.12 b	4.52 ± 0.30
Mean	2.23	4.56	5.90	4.27
F-Test	ns	ns	*	ns
C.V.%	5.96	19.34	11.69	16.69

* = Significant at $P \leq 0.05$; ** = highly significant at $P \leq 0.01$, and ns=non-significant at $P > 0.05$.

4.5 Discussion

4.5.1 Genotypic, environmental, and genotype-by-environment interaction effects on nutritional composition

The proximate nutritional composition of mungbean seeds and sprouts was significantly influenced by G, E, and GEI, as confirmed by combined analysis of variance (Tables 4.2 and 4.3). Most measured traits, moisture, crude protein, crude fat, total ash, crude fiber, and carbohydrate, exhibited considerable variability, emphasizing the combined role of genetic background and environmental conditions in shaping nutritional outcomes.

Moisture content in seeds and sprouts displayed strong environmental sensitivity. Seeds harvested from the rainy-season site (PNR) exhibited significantly higher moisture content than those from the dry-season site (CND), likely due to higher relative humidity and prolonged field exposure before harvest. Seed moisture content ranged from 8.06-11.44%, within the recommended 8.00-12.00% range for prolonged storage and viability (Irfan et al., 2022). In contrast, mungbean sprouts showed higher moisture levels under CND conditions. Elevated moisture in sprouts may reduce shelf life by promoting microbial proliferation, increased respiration, or tissue degradation (Chávez-García et al., 2023). Therefore, PNR-grown sprouts, which had relatively lower moisture content, may offer better postharvest stability.

Crude protein content was largely determined by G but strongly modulated by E. In seeds, check varieties CN3 and CN84-1 consistently showed higher protein levels, highlighting their superior adaptability and nitrogen assimilation capacity. Among the new lines, D5 (with SUT1 as the recurrent parent) exhibited the highest protein content under CND, suggesting a favorable response to nitrogen-enriched soils. Protein content was significantly higher in CND than in PNR, likely reflecting the more fertile clay loam soil in CND, with higher organic matter and nitrogen levels (Table 1). These findings align with Malik et al. (2003), who demonstrated that nitrogen enrichment enhances mungbean seed protein. Adequate nitrogen promotes amino acid synthesis and protein accumulation, while deficiencies limit protein deposition (Uchida, 2000; Azadi et al., 2013; Ge et al., 2024). In sprouts, the new lines, P08, P12, P22, and P24, showed lower protein levels than CN84-1, mirroring seed trends. The reduced protein content in resistant lines may be due to reallocation of metabolic resources toward defense-related proteins such as β-1,3-glucanase and peroxidase, particularly in lines bred for CLS and PM resistance (Inthaisong et al.,

2025). Nevertheless, seed and sprout protein contents observed (22.40–32.35%) were slightly higher than many other common legumes (Li et al., 2010; Gunathilake et al., 2016; Yi-Shen et al., 2018; Idris et al., 2025) and comparable to kidney beans (20.00–30.00%) (Shevkani et al., 2015), cowpea (22.80-25.20%) (Gunathilake et al., 2016), chickpea (19.30–21.00%) (Dahiya et al., 2013), affirming mungbean's role as a protein-dense legume (Anwar et al., 2007).

Crude fat content in seeds and sprouts showed genotypic variation, although the absolute differences were small. Lines P12 and P22 consistently exhibited higher fat levels than other genotypes across both environments. Fat content in seeds ranged from 0.42–1.22%, and in sprouts from 0.86–1.84%. Despite statistical significance, these narrow ranges suggest limited genetic divergence for this trait. P08 consistently exhibited the lowest crude fat in both seeds and sprouts, aligning with consumer preference for low-fat plant-based foods.

Total ash content, representing the mineral composition of plant tissues, varied modestly among genotypes. In seeds, no consistent differences were found between new lines and check varieties across environments. However, in sprouts, line P24 exhibited significantly higher average ash content across both environments than other genotypes, suggesting greater mineral deposition efficiency. Ash consists primarily of essential minerals like calcium, potassium, and sodium (Shokunbi et al., 2023), and high values are nutritionally advantageous. However, overly high ash levels may indicate contamination from soil particles or processing agents (Marshall, 2010), warranting further quality control.

Crude fiber content was primarily governed by G, with notable interactions with the environment. In seeds, P12 displayed the highest average fiber levels, while P22 and P24 had values comparable to CN84-1. P08 and D5 exhibited lower fiber levels, similar to CN3. In sprouts, line P22 showed the highest average fiber content across both environments. High fiber content enhances food texture and contributes to satiety. Cellulose and hemicellulose, key components of crude fiber, may improve crispness and moisture balance in food products (Zdunek et al., 2014; Trandel-Hayse, 2023), offering functional food advantages for high fiber mungbean lines.

Carbohydrate content was strongly influenced by G and E. In both seeds and sprouts, lines P08 and P24 consistently exhibited the highest average carbohydrate levels across environments. Seeds from PNR showed higher carbohydrate content than those from CND, possibly due to altered starch accumulation under high

humidity during pod filling. Carbohydrate accumulation is known to be sensitive to environmental stress, carbohydrate metabolism and accumulation are highly dynamic and respond to various abiotic stresses. These stresses can increase or decrease the concentration of soluble sugars and starch in different plant organs, depending on the type of stress and plant genotype (Rosa et al., 2009; Sehgal et al., 2018), and these findings affirm the role of E and GEI in shaping this trait. Mungbean seeds contained 50.00–60.00% carbohydrates, higher than soybeans (~35%) (Hou et al., 2019), highlighting their potential in starch-based processing. However, carbohydrate content in sprouts was generally lower than in seeds, likely due to metabolic conversion during sprouting. This reduction benefits individuals managing blood sugar levels, while higher carbohydrate sprouts may support energy-intensive needs, such as athletes. Sprouting also enhances antioxidant concentrations and nutrient bioavailability (Tang et al., 2014). Carbohydrate levels are also influenced by the composition of other macronutrients, often increasing when protein levels are low, as seen in the newly developed lines. Specifically, the new lines P08 and P24 demonstrated high carbohydrate content, but conversely, exhibited relatively low protein levels. On the other hand, varieties CN3 and CN84-1, showed a distinct profile with higher protein content but lower carbohydrate levels. This pattern aligns with previous research indicating that traditional varieties tend to have higher protein concentrations, which may be more suitable for nutritional applications where protein is the main focus.

4.5.2 Integrated nutritional response and breeding implications

The combined analysis revealed that GEI significantly influenced all evaluated nutritional traits in both mungbean seeds and sprouts. While genotypes emerged as the predominant factor determining the compositional quality of most traits. Environmental factors, including rainfall distribution, temperature regimes, soil fertility, and nutrient availability, acted as critical modulators of nutrient expression. For instance, the CND environment, characterized by higher nitrogen availability, elevated levels of organic matter and potassium, and relatively stable growing conditions, promoted enhanced accumulation of protein and ash. In contrast, the PNR environment, which was more humid, favored higher moisture and carbohydrate contents, likely due to increased water retention and environmental induced carbohydrate synthesis. The presence of significant GEI effects also indicated that genotypic performance varied across environments, as evidenced by the differing

rank orders of nutritional traits. This variability underscores the importance of GEI in guiding breeding efforts and cultivation practices to optimize specific nutritional traits.

Notably, certain genotypes exhibited distinct nutritional advantages: P08 and P24 consistently accumulated higher carbohydrate levels; P12 and P22 were superior in seed and sprout fiber content, respectively; and D5 demonstrated enhanced protein content under favorable environmental conditions. These genotype-specific strengths reflect their potential utility in targeted nutritional improvement and industrial applications. Moreover, the adaptability of these lines across divergent environments highlights their promise for the development of climate-resilient, nutrient-rich mungbean varieties.

4.5.3 Root length and drought adaptation

The morphological traits of seven mungbean genotypes revealed significant variation in root length, while no significant differences were observed in hypocotyl diameter, hypocotyl length, and output ratio. Genotypes CN84-1 and P24 exhibited greater root length. Root architecture is a critical determinant of early legume seedling performance, particularly under drought stress (Wang et al., 2024; Afonso et al., 2025). Drought-tolerant legume genotypes typically possess increased total root length, higher root density, and deeper root penetration, facilitating more efficient water uptake during soil moisture deficit periods (Khatun et al., 2021; Wang et al., 2024). Especially during the early germination stage, mungbean genotypes with longer roots tend to exhibit better drought tolerance. Several studies have confirmed that mungbean varieties with more developed root systems demonstrate greater drought tolerance and improved physiological traits under stress, including higher relative water content, enhanced membrane stability, and superior yield performance (Bangar et al., 2019; Khan et al., 2025). Nonetheless, additional research is necessary to validate these results.

4.5.4 Hypocotyl dynamics and seedling vigor

Although differences in hypocotyl length were not statistically significant, a trend toward longer hypocotyls was observed, which may correlate with more robust seedling establishment. This observation aligns with the findings of Yu and Huang (2017), who reported that hypocotyl length changes markedly during early plant growth, particularly between seed germination and seedling establishment, highlighting the importance of this trait for successful emergence. While such differences may not always reach statistical significance, longer hypocotyls have been associated with

improved establishment in various plant species and are likely relevant in legumes as well. This trait may contribute to enhanced light competition and mechanical stability during early developmental stages. However, further studies are required to confirm this finding.

4.5.5 Morphological traits and commercial efficiency in mungbean sprout production

The efficiency and marketability of mungbean sprouts are strongly influenced by morphological traits such as hypocotyl diameter, hypocotyl length, root length, and output ratio. Commercially desirable sprouts were typically thick, crisp, and uniform, with a hypocotyl length of 3.0–7.0 cm, as these attributes enhanced texture, appearance, and consumer appeal (Shanmugasundaram, 2007; Gatbonton et al., 2022). High-quality mungbean sprouts were characterized by thick hypocotyls and short roots. The experimental results indicated that these traits varied among genotypes, although all genotypes showed relatively similar characteristics suitable for sprout production and only minor differences, particularly in root length. Notably, line D5 exhibited short roots and tended to have a high output ratio. Short roots were preferred because they simplified cleaning and packaging, while adequate root development supported vigorous sprout growth. However, root length could be shortened through the application of growth regulators. For instance, (Chen et al., 1987) reported that the application of ethephon at concentrations of 10–20 ppm, either as a single or double application, significantly improved sprout quality by reducing root length and increasing hypocotyl thickness. The output ratio was crucial for producer profitability and was associated with vigorous seedling growth and high germination rates. Producers selected mungbean varieties and optimized environmental conditions to increase these traits, thereby ensuring efficient production and meeting market standards. In summary, selecting genotypes with optimal hypocotyl and root traits and high output ratios was essential for producing high-quality, market-preferred mungbean sprouts.

Overall, in terms of nutritional values based on the proximate compositions, CN84-1 emerges as the most suitable mungbean variety for producing sprouts with a high protein content, catering to consumers seeking protein-enriched diets. In contrast, P24 and P08 are preferable choices for sprout production when a higher carbohydrate content and a more desirable taste, often associated with sweetness and appealing mouthfeel, are desired. However, higher carbohydrates may lead to a shorter shelf life compared to genotypes with lower levels, as carbohydrates provide a readily available

substrate for microbial growth, potentially accelerating spoilage. For applications where an elevated dietary fiber content is the priority, P22 would be the optimal choice, offering increased nutritional benefits related to digestive health. These findings underscore that the selection of mungbean variety can be strategically tailored according to the targeted nutritional profile, desired sensory characteristics, and specific end-use applications of the sprouts, balancing nutritional benefits with practical considerations such as shelf life.

4.6 Conclusions

This study comprehensively evaluated the proximate nutritional composition of seven mungbean genotypes, including five newly developed lines, and two check varieties, grown under two contrasting environments (PNR and CND). Significant effects of G, E, and GEI were observed for most nutritional traits. Environmental factors such as humidity, temperature, and soil nutrient content influenced nutrient accumulation. PNR favored higher moisture, fat, and carbohydrate contents, while CND enhanced protein and ash levels. GEI effects indicated environment-dependent genotypic performance, supporting the need for targeted breeding and cultivation strategies. Notably, P08 and P24 exhibited high carbohydrate content, P12 and P22 were rich in seed and sprout fiber, and D5 showed superior protein accumulation under favorable conditions. Although the check varieties CN3 and CN84-1 maintained high protein levels, some new lines outperformed them in specific traits. Among morphological characteristics, root length emerged as a discriminating trait among genotypes. These findings highlight the complex interaction of G, E, and GEI in shaping mungbean nutritional quality. The strong performance of several new lines, some exceeding check varieties in key nutrients, underscores their potential to enhance the nutritional, industrial, and agronomic value of mungbean in sustainable agricultural systems.

4.7 References

Afonso, P., Castro, I., Couto, P., Leal, F., Carnide, V., Rosa, E., & Carvalho, M. (2025). Root phenotyping: a contribution to understanding drought stress resilience in grain legumes. *Agron., 15*(4), 798. doi:10.3390/agronomy15040798

Anwar, F., Latif, S., Przybylski, R., Sultana, B., & Ashraf, M. (2007). Chemical composition and antioxidant activity of seeds of different cultivars of mungbean. *J. Food Sci., 72*(7), S503-S510. doi:10.1111/j.1750-3841.2007.00462.x

AOAC. (2019). *Official methods of analysis*. 21sted. AOAC Publishing: Washington, DC, USA: Association of Official Analytical Chemists.

Asaro, A., Ziegler, G., Ziyomo, C., Hoekenga, O. A., Dilkes, B. P., & Baxter, I. (2016). The interaction of genotype and environment determines variation in the maize kernel ionome. *G3, 6*(12), 4175-4183. doi:10.1534/g3.116.034827

Azadi, E., Rafiee, M., & Nasrollahi, H. (2013). The effect of different nitrogen levels on seed yield and morphological characteristic of mungbean in the climate condition of Khorramabad. *Biol. Res., 4*(2), 51–55.

Bangar, P., Chaudhury, A., Tiwari, B., Kumar, S., Kumari, R., & Bhat, K. V. (2019). Morphophysiological and biochemical response of mungbean [*Vigna radiata* (L.) Wilczek] varieties at different developmental stages under drought stress. *Turk. J. Biol., 43*(1), 58-69. doi:10.3906/biy-1801-64

Bhatty, N., Gilani, A. H., & Ahmad, N. S. (2000). Nutritional value of mung bean (*Vigna radiata*) as effected by cooking and supplementation. *Arch. Latinoam. Nutr., 50*(4), 374-379.

Chaiyapan, C., Khairum, A., Chueakhunthod, W., Pookhamsak, P., Siwapithakpong, K., & Tantasawat, P. A. (2023). In *Vitro* selection of mungbean genotypes for drought tolerance by polyethylene glycol induced water deficit. *Chiang Mai J. Sci., 50*(3), 1-11. doi:10.12982/CMJS.2023.035

Chávez-García, S. N., Rodríguez-Herrera, R., Flores, S. N., Silva-Belmares, S. Y., Esparza-González, S. C., Ascacio-Valdés, J. A., & Flores-Gallegos, A. C. (2023). Sprouts as probiotic carriers: a new trend to improve consumer nutrition. *Food Chem.: Mol. Sci., 7*(100185). doi:10.1016/j.fochms.2023.100185

Chen, S.-l., Breene, W. M., & Schowalter, C. (1987). Effects of growth regulators on yield and quality of mungbean sprouts grown in an automatically controlled chamber. *J. Food Qual., 10*(4), 219-238. doi:10.1111/j.1745-4557.1987.tb00814.x

Cong, B., Maxwell, C., Luck, S., Vespestad, D., Richard, K., Mickelson, J., & Zhong, C. (2015). Genotypic and environmental impact on natural variation of nutrient

composition in 50 non genetically modified commercial maize hybrids in North America. *J. Agric. Food Chem., 63*(22), 5321-5334. doi:10.1021/acs.jafc.5b01764

Dahiya, P. K., Linnemann, A. R., Nout, M. J. R., van Boekel, M. A. J. S., & Grewal, R. B. (2013). Nutrient composition of selected newly bred and established mung bean varieties. *LWT., 54*(1), 249-256. doi:10.1016/j.lwt.2013.05.017

Department of Agriculture. (2018). *Data recording manual for recording mungbean research data*. Retrieved from www.doa.go.th/fc/chainat/wp-content/uploads/2020/06/คู่มือการบันทึกข้อมูลงานวิจัยถั่วเขียว.pdf

Fathonah, S., Rosidah, R., Iswari, R. S., Amalia, B., & Humaizah, S. (2023). The acceptability, expiration, and fibre level of gluten-free mung bean biscuits. *Food Res., 7*(2), 262-271. doi:10.26656/fr.2017.7(2).769

Gatbonton, M. C. C., Molon, C. R., Agatep, R. C., & Beato, L. L. (2022). Mungbean sprout production in Calabarzon region, Philippines. *Int. J. Adv. Res., 10*(3), 904-911. doi:10.21474/IJAR01/14469

Ge, J., Du, Y., Wang, Q., Xu, X., Li, J., Tao, J., . . . Gao, J. (2024). Effects of nitrogen fertilizer on the physicochemical, structural, functional, thermal, and rheological properties of mung bean (*Vigna radiata*) protein. *Int. J. Biol. Macromol., 260*, 129616. doi:10.1016/j.ijbiomac.2024.129616

Gore, P. G., Das, A., Bhardwaj, R., Tripathi, K., Pratap, A., Dikshit, H. K., . . . Gupta, V. (2021). Understanding g × e interaction for nutritional and antinutritional factors in a diverse panel of *Vigna stipulacea* (lam.) kuntz germplasm tested over the locations. *Frontiers in Plant Science, 12*. doi:10.3389/fpls.2021.766645

Gunathilake, K. T., Herath, T., & Wansapala, J. (2016). Comparison of physicochemical properties of selected locally available legume varieties (mung bean, cowpea and soybean). *Potr. S. J. F. Sci., 10*(1), 424-430. doi:10.5219/631

Hailu, K. H. (2018). Determination of proximate composition and bioactive compounds of the Abyssinian purple wheat. *Cogent food agric., 4*(1), 415-421.

Hou, D., Yousaf, L., Xue, Y., Hu, J., Wu, J., Hu, X., . . . Shen, Q. (2019). Mung bean (*Vigna radiata* L.): bioactive polyphenols, polysaccharides, peptides, and health benefits. *Nutr., 11*(6), 1238. doi:10.3390/nu11061238

Idris, F. M., Urga, K., Admassu, H., Fentie, E. G., Kwon, S.-M., & Shin, J.-H. (2025). Profiling the nutritional, phytochemical, and functional properties of mung bean varieties. *Foods, 14*(4), 571. Retrieved from https://www.mdpi.com/2304-8158/14/4/571

Irfan, M., Bhat, M. A., Rashid, U., Bhat, F. A., & Alwutayd, K. M. (2025). Mungbean G × E interaction unveiling resistance to Cercospora leaf spot through GGE biplot analysis. *Sci. Rep., 15*(1), 15368. doi:10.1038/s41598-025-98885-1

Inthaisong, S., Boonchuen, P., Jaichopsanthia, T., Songwattana, P., Khairum, A., Chueakhunthod, W., . . . Tantasawat, P. A. (2025). Insights into mungbean defense response to Cercospora leaf spot based on transcriptome analysis. *Sci. Rep., 15*(1), 1334. doi:10.1038/s41598-024-84787-1

Jomsangawong, A., Masari, A., Phruetthithep, C., Bunsak, C., Chaiwan, P., Pankaw, W., . . . Phoomthaisong, J. (2022). *Mungbean variety "CHAI NAT 3"*. Department of Agriculture, Ministry of Agriculture and Cooperatives. Retrieved from https://www. doa.go.th/research/attachment.php?aid=2944

Karami, Z., & Duangmal, K. (2024). Exploring the effect of incubation pH, temperature, and in vitro digestion on antioxidant potential of mung bean protein hydrolysates. *Chiang Mai J. Sci., 51*(6). doi:10.12982/CMJS.2024.090

Khan, A. A., Wang, Y. F., Akbar, R., & Alhoqail, W. A. (2025). Mechanistic insights and future perspectives of drought stress management in staple crops. *Front. Plant Sci., 16*. doi:10.3389/fpls.2025.1547452

Khatun, M., Sarkar, S., Era, F. M., Islam, A. K. M. M., Anwar, M. P., Fahad, S., . . . Islam, A. K. M. A. (2021). Drought stress in grain legumes: effects, tolerance mechanisms and management. *Agron., 11*(12), 2374. Retrieved from https://www.mdpi.co m/2073-4395/11/12/2374

Levesque, R. (2007). *A guide for SPSS and SAS users: SPSS programming and data management*. 2. Retrieved from Retrieved from https://www.spsstools.net/en/documents/74/SPSS_Programming_and_Data_Management_2nd_edition.pdf

Li, W., Shu, C., Yan, S., & Shen, Q. (2010). Characteristics of sixteen mung bean cultivars and their protein isolates. *Int. J. Food Sci. Technol., 45*(6), 1205-1211. doi:10.1111/j.1365-2621.2010.02259.x

Malik, M. A., Saleem, M. F., Ali, A., & Mahmood, I. (2003). Effect of nitrogen and phosphorus application on growth yield and quality of mungbean (*Vigna radiata* L.). *Pak. J. Agric. Sci., 40*, 133-136.

Marshall, M. R. (2010). Ash analysis. In *Food analysis* (pp. 105-115). doi:10.1007/978-1-4419-1478-1_7

Masari, A., Ngampongsai, S., Bunsak, C., Chaiwan, P., & Thanomsub, S. (2016). Development of mungbean processed products at the household enterprise level. *Thai Agric. Res. J., 34*(1), 95-106. doi:10.14456/thaidoa-agres.2016.2

Napier, J. D., Heckman, R. W., & Juenger, T. E. (2023). Gene-by-environment interactions in plants: Molecular mechanisms, environmental drivers, and adaptive plasticity. *Plant Cell, 35*(1), 109-124. doi:10.1093/plcell/koac322

Papan, P., Chueakhunthod, W., Khairum, A., Siwapitakpong, K., Chaiyapan, C., Inthaisong, S., . . . Tantasawat, P. A. (2024). Marker-assisted gene pyramiding for powdery mildew resistance in Thai mungbean variety SUT1 by backcross breeding. *Plant Mol. Biol. Rep.* doi:10.1007/s11105-024-01445-6

Pregitzer, C. C., Bailey, J. K., & Schweitzer, J. A. (2013). Genetic by environment interactions affect plant-soil linkages. *Ecol. Evol., 3*(7), 2322-2333. doi:10.1002/ece3.618

Randhir, R., & Shetty, K. (2005). Developmental stimulation of total phenolics and related antioxidant activity in light-and dark-germinated corn by natural elicitors. *Process Biochem., 40*(5), 1721-1732. doi:10.1016/j.procbio.2004.06.064

Rosa, M., Prado, C., Podazza, G., Interdonato, R., González, J. A., Hilal, M., & Prado, F. E. (2009). Soluble sugars-metabolism, sensing and abiotic stress: a complex network in the life of plants. *Plant Signal Behav., 4*(5), 388-393. doi:10.4161/psb.4.5.8294

Sehgal, A., Sita, K., Siddique, K. H. M., Kumar, R., Bhogireddy, S., Varshney, R. K., . . . Nayyar, H. (2018). Drought or/and heat-stress effects on seed filling in food crops: impacts on functional biochemistry, seed yields, and nutritional quality. *Front. Plant Sci., 9*. doi:10.3389/fpls.2018.01705

Shanmugasundaram, S. (2007). Exploit mungbean with value-added products (pp. 99-102). Belgium, Leuven

Shevkani, K., Singh, N., Kaur, A., & Rana, J. C. (2015). Structural and functional characterization of kidney bean and field pea protein isolates: a comparative study. *Food Hydrocoll., 43*, 679-689. doi:10.1016/j.foodhyd.2014.07.024

Shokunbi, O. S., Adepoju, O. T., Ramaite, I. D. I., Shokunbi, O. S., Mojapelo, P. E. L., & Akinyele, I. O. (2023). Potassium, sodium, calcium and magnesium levels of commonly consumed foods and estimates of dietary intakes of selected Nigerian adults. *Heliyon, 9*(3). doi:10.1016/j.heliyon.2023.e13729

Somta, P., Laosatit, K., Yuan, X., & Chen, X. (2022). Thirty years of mungbean genome research: where do we stand and what have we learned? *Front. Plant Sci., 13*. doi:10.3389/fpls.2022.944721

Tang, D., Dong, Y., Ren, H., Li, L., & He, C. (2014). A review of phytochemistry, metabolite changes, and medicinal uses of the common food mung bean and its sprouts (*Vigna radiata*). *Chem. Cent. J., 8*(1), 4. doi:10.1186/1752-153x-8-4

Trandel-Hayse, M. (2023). *A deeper look into blueberry cell wall composition and fruit firmness phenotypes*. Retrieved from https://www.vacciniumcap.org/blueberr ycell wallcomp?utm

Uchida, R. (2000). *Essential nutrients for plant growth: nutrient functions and deficiency symptoms*. *Plant nutrient management in Hawaii's soils.* Retrieved from https://www.ctahr.hawaii.edu/oc/freepubs/pdf/pnm3.pdf

Vaidya, P., & Stinchcombe, J. R. (2020). The potential for genotype-by-environment interactions to maintain genetic variation in a model legume–rhizobia mutualism. *Plant Commun., 1*(6), 100114. doi:10.1016/j.xplc.2020.100114

Wang, Huang, M., Yang, S., Li, X., Gao, Y., Yang, P., . . . Gao, X. (2021). Study on nutritional characteristics and antioxidant capacity of mung bean during germination. *Czech J. Food Sci., 39*(6), 469-478.

Wang, Z., Yung, W. S., Gao, Y., Huang, C., Zhao, X., Chen, Y., . . . Lam, H. M. (2024). From phenotyping to genetic mapping: identifying water-stress adaptations in legume root traits. *BMC Plant. Biol., 24*(1), 749. doi:10.1186/s12870-024-05477-8

Yeap, S. K., Mohd, A. N., Mohd, Y. H., Alitheen, N. B., Beh, B. K., Ho, W. Y., . . . Long, K. (2012). Antihyperglycemic effects of fermented and nonfermented mung bean extracts on alloxan-induced-diabetic mice. *Biomed Res. Int., 2012*(1), 285430. doi:10.1155/2012/285430

Yeremko, L., Czopek, K., Staniak, M., Marenych, M., & Hanhur, V. (2025). Role of environmental factors in legume-*Rhizobium* symbiosis: a review. *Biomolecules, 15*(1). doi:10.3390/biom15010118

Yu, Y., & Huang, R. (2017). Integration of ethylene and light signaling affects hypocotyl growth in arabidopsis. *Front. Plant Sci., 8*. doi:10.3389/fpls.2017.00057

Zdunek, A., Kozioł, A., Pieczywek, P. M., & Cybulska, J. (2014). Evaluation of the nanostructure of pectin, hemicellulose and cellulose in the cell walls of pears of different texture and firmness. *Food Bioproc. Tech., 7*(12), 3525-3535. doi:10.1007/s11947-014-1365-z