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RESEARCH ARTICLE

Identification of morphological traits affecting high seed yield potential from new hemp germplasm collected in Thailand

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ABSTRACT

Hemp (*Cannabis sativa* L.) seeds, used as raw materials in the food and cosmetic industries globally, require high seed-producing varieties, as most developed ones focus on medical purposes. This study identified morphological traits affecting high seed yield in local hemp accessions collected from 63 locations across 11 districts in three northern Thailand provinces. The 37 surviving accessions were evaluated, clustered based on morphological data, and compared with hemp varieties RPF1 and RPF2. Cluster analysis classified these into five clusters, with Cluster 1 having the highest seed weight per plant (840.68 g) and longest inflorescence (11.25 cm). First-year correlation analysis revealed a significant positive relationship ($p \leq 0.01$) between seed weight per plant and inflorescence length (0.50) and stem diameter (0.46). In the second year, the number of flowers per inflorescence significantly correlated with seed weight per plant (0.55). Path coefficient analysis showed a high direct effect on seed yield from inflorescence length (0.37) and stem diameter (0.32) in the first year and branch length (0.80), height (0.73), and number of flowers per inflorescence (0.72) in the second year. Moreover, 24 hemp lines from the second year with seed yield potential were selected for the hemp breeding program. Morphological traits directly affecting seed yield can be used as selection criteria in the hemp breeding programs.

Key words: *Cannabis sativa*, hemp, breeding, direct effect, path coefficient analysis, seed yield, selection criterion.

INTRODUCTION

Hemp (*Cannabis sativa* L.) is a versatile plant with a wide range of applications across numerous industries, including cosmetics, food, medicine, antimicrobial chemicals, construction materials, energy, textiles, automobiles, paper, and more. Almost every part of the hemp plant, such as stems, seeds, leaves, and flowers, finds use in these sectors (Chaowana et al., 2024; Yimlamai et al., 2024). Hemp seeds are particularly noteworthy due to their rich content of proteins, essential fatty acids, fibers, vitamins, and minerals. They are often incorporated as supplements in a variety of food products (Cerino et al., 2021). Recently, the health benefits of hemp seeds have led to their increased popularity in the food industry. They are an excellent source of plant-based proteins, providing all nine essential amino acids, thus qualifying them as a complete protein source. Additionally, they are high in omega-3 and omega-6 fatty acids, which are crucial for brain and heart health (Siano et al., 2018).

The hemp seed industry requires varieties that produce high seed yields. However, most existing hemp varieties have been genetically enhanced for their phytochemical content, such as cannabinoids, terpenes, and flavonoids (Farag and Kayser, 2017). This creates a demand for new hemp varieties with high seed yield potential. The selection of hemp varieties for seed production should be based on factors related to seed yield, including seed weight per plant, 100-seed weight, plant height, number of branches, stem diameter, number of flowers per plant, and flower length (Abdollahi et al., 2020). These characteristics play a critical role in determining seed yield potential (Abdollahi et al., 2020).

The breeding of hemp varieties with high seed yield potential is a top priority for hemp breeding programs. Hemp genetic materials from Canada, China, Europe, India, Russia, and now Thailand have been collected and utilized in these efforts. Notably, Chinese and Indian hemp germplasm are primarily used for their flowers and phytochemicals. Russian genetic materials are known for their exceptional seed yield and oil content, while Canadian and European genetic materials are prized for their fiber quality (Sawler et al., 2015). In Thailand, hemp cultivated in the northern regions is used for fiber production. The hemp varieties developed in Thailand have primarily focused on fiber production. However, as the food industry requires hemp seeds, the hemp varieties for fiber production are also used for seed production. This necessitates the development of new hemp varieties specifically for high seed yields. Before incorporating genetic material into breeding programs, it must be evaluated in plants that exhibit the target traits. Path coefficient analysis can be employed for the evaluation of genetic material, helping to identify traits that significantly impact seed yield (Thongthip et al., 2023).

Pod and seed yield are quantitative traits that are significantly influenced by environmental factors (Pobkhunthod et al., 2022). These yields can also be significantly impacted by other yield components, which serve as valuable indicators for genotype selection (Khan et al., 2022). Path coefficient analysis, a statistical method used to determine the direct and indirect effects of variables on a specific outcome, has been successfully employed in identifying crucial yield traits in various crops, including bambara groundnut, chili, lentil, mung bean, and wheat (Mekonnen et al., 2014; Usman et al., 2017; Khan et al., 2022). Traits that exert a strong influence on hemp seed yield can provide valuable insights and aid in hemp breeding in tropical regions.

The growing demand for hemp seeds in the food industry necessitates the use of hemp varieties with high seed production potential. However, the cannabis varieties cultivated in Thailand and surrounding regions were primarily developed for fiber production. As a result, this region lacks hemp varieties specifically bred for seed production. The primary focus of the present study was to evaluate the collected hemp accessions for seed yield potential and agronomic characteristics. Furthermore, agronomic traits affecting seed yield potential were identified using path coefficient analysis as criteria for future breeding of high seed-yield hemp varieties.

MATERIALS AND METHODS

Hemp seed collection

Hemp (*Cannabis sativa* L.) samples utilized in the study were collected from local planting sites in northern Thailand (Table 1). Seeds were obtained from exceptional hemp plants at each location, and their 100-seed weight, length, and width were documented. The gathered hemp accessions served as the primary plant material for estimating phenotypic variations in comparison to two hemp varieties, RPF1 and RPF2 (control varieties). These two varieties have been predominantly used for seed and biomass production in northern Thailand. Hemp accessions with potential for seed yield were selected for comparison of agronomic traits. The study was conducted in an experimental field in Pang Mu (19°21'04" N, 97°57'30" E), Mae Hong Son, Thailand.

Selection of outstand hemp accessions for yield potential

In the first year, hemp seeds were sown in seedling pots to determine their germination rate. After a month of germination, the seedlings were transplanted into the experimental field. Forty seedlings were selected to represent each accession. In the experimental field, each accession was transplanted into four rows, each 5 m long, with a row-to-row spacing of 150 cm and a plant-to-plant spacing of 80 cm. The prescribed fertilizer, comprising 15 kg each of N, P, and K, was applied as the basal dose post transplantation.

Phenotypic and agronomic traits of each hemp accession were registered at the harvesting stage. The accessions were considered to be at the harvesting stage when approximately 70%-80% of the bracts around the seed were ripe and visible. The following traits were recorded postharvest: Plant height (cm), number of branches per plant, stem diameter (mm), number of flowers on an inflorescence, inflorescence length (cm), trichome density score, weight of 100 seeds (g), and seed weight per plant (g). The trichome density score for each accession was evaluated visually using a method adapted from Shahzad et al.

(2021). The hemp accessions were scored for trichome density on a scale of 1 to 5 (1 = extremely low density, 2 = low density, 3 = moderate density, 4 = high density, and 5 = extremely high density). The data on yield and yield components were used for grouping the hemp accessions, correlation, and path coefficient analysis.

Table 1. Origin of the 63 hemp landraces collected from the North of Thailand and their seed characteristics.

Code	Place	District	Province	100 seed weight	Seed width	Seed length
				g	mm	mm
KU001	Samoeng Tai	Samoeng	Chiang Mai	3.01	3.49	5.20
KU002	Samoeng Tai	Samoeng	Chiang Mai	3.22	3.45	5.09
KU003	Samoeng Tai	Samoeng	Chiang Mai	3.38	3.59	4.67
KU004	Samoeng Tai	Samoeng	Chiang Mai	2.95	3.67	4.94
KU005	Samoeng North	Samoeng	Chiang Mai	3.66	4.00	5.36
KU006	Samoeng North	Samoeng	Chiang Mai	3.17	3.80	4.93
KU007	Samoeng North	Samoeng	Chiang Mai	2.18	3.57	4.76
KU008	Mae Ramat	Mae Ramat	Tak	3.31	3.89	4.94
KU009	Mae Ramat	Mae Ramat	Tak	2.90	3.55	4.67
KU010	Mae Charao	Mae Ramat	Tak	2.60	3.69	5.14
KU011	Mae Tuen	Mae Ramat	Tak	2.71	4.04	5.38
KU012	Sam Muen	Mae Ramat	Tak	2.92	3.97	5.18
KU013	Phra That	Mae Ramat	Tak	2.41	3.22	4.76
KU014	Khiri Rat	Phop Phra	Tak	3.04	3.37	4.55
KU015	Khiri Rat	Phop Phra	Tak	1.62	3.83	5.03
KU016	Phop Phra	Phop Phra	Tak	1.02	2.91	4.57
KU017	Phop Phra	Phop Phra	Tak	3.42	3.56	4.86
KU018	Phop Phra	Phop Phra	Tak	2.95	3.56	4.74
KU019	Phop Phra	Phop Phra	Tak	2.63	3.54	4.40
KU020	Chong Khaep	Phop Phra	Tak	1.89	3.39	4.87
KU021	Chong Khaep	Phop Phra	Tak	3.09	3.36	4.95
KU022	Chong Khaep	Phop Phra	Tak	1.80	3.22	4.72
KU023	Na Bot	Wang Chao	Tak	2.11	3.47	3.85
KU024	Mae Sot	Mae Sot	Tak	2.43	3.61	4.70
KU025	Mae Sot	Mae Sot	Tak	2.91	3.39	4.57
KU026	Mae Sot	Mae Sot	Tak	2.63	3.26	4.94
KU027	Tha Sai Luat	Mae Sot	Tak	3.49	3.61	5.32
KU028	Tha Sai Luat	Mae Sot	Tak	3.77	3.51	5.03
KU029	Mae Tao	Mae Sot	Tak	3.02	3.50	4.94
KU030	Mae Tao	Mae Sot	Tak	3.07	3.77	5.05
KU031	Mae Tao	Mae Sot	Tak	2.66	3.67	5.18
KU032	Mae Tuen	Mae Ramat	Tak	2.69	3.17	4.90
KU033	Mae Tuen	Mae Ramat	Tak	2.45	3.45	4.68
KU034	Mae Tuen	Mae Ramat	Tak	3.67	3.77	4.89
KU035	Mae Tuen	Mae Ramat	Tak	2.11	3.57	3.91
KU036	Mae Tuen	Mae Ramat	Tak	2.65	3.69	5.08
KU037	Mae Tuen	Mae Ramat	Tak	2.72	3.51	5.58
KU038	Mae Tuen	Mae Ramat	Tak	2.62	3.45	4.00
KU039	Khun Yuam	Khun Yuam	Mae Hong Son	2.42	3.06	4.68
KU040	Khun Yuam	Khun Yuam	Mae Hong Son	3.05	3.62	4.83
KU041	Khun Yuam	Khun Yuam	Mae Hong Son	2.44	3.44	4.77
KU042	Khun Yuam	Khun Yuam	Mae Hong Son	3.67	3.47	5.50
KU043	Mae Yuam Noi	Khun Yuam	Mae Hong Son	1.33	2.80	4.02
KU044	Mae Yuam Noi	Khun Yuam	Mae Hong Son	2.20	3.74	4.98
KU045	Mae Yuam Noi	Khun Yuam	Mae Hong Son	2.62	3.49	5.00
KU046	Mueang Pon	Khun Yuam	Mae Hong Son	3.39	3.38	4.97
KU047	Mueang Pon	Khun Yuam	Mae Hong Son	3.54	2.84	5.09
KU048	Mueang Pon	Khun Yuam	Mae Hong Son	2.96	3.54	4.88
KU049	Mae Ngao	Khun Yuam	Mae Hong Son	3.35	3.38	5.36
KU050	Sop Moei	Sop Moei	Mae Hong Son	1.85	3.27	4.84
KU051	Sop Moei	Sop Moei	Mae Hong Son	2.61	3.26	4.87
KU052	Mae Suat	Sop Moei	Mae Hong Son	2.85	3.14	4.95
KU053	Mae Yuam	Mae Sariang	Mae Hong Son	3.05	3.54	4.90
KU054	Mae Yuam	Mae Sariang	Mae Hong Son	0.94	2.16	3.55
KU055	Mae Sariang	Mae Sariang	Mae Hong Son	1.74	2.84	4.33
KU056	Mae Sariang	Mae Sariang	Mae Hong Son	1.68	2.79	4.10
KU057	Ban Kat	Mae Sariang	Mae Hong Son	1.86	2.90	4.45
KU058	Ban Kat	Mae Sariang	Mae Hong Son	1.97	2.94	4.57
KU059	Huai Pha	Mueang	Mae Hong Son	1.68	2.36	3.17
KU060	Mae La Noi	Mae La Noi	Mae Hong Son	1.87	3.29	4.56
KU061	Sop Pong	Pang Mapha	Mae Hong Son	1.49	2.84	4.12
KU062	Pang Mapha	Pang Mapha	Mae Hong Son	1.72	2.74	4.29
KU063	Pang Mapha	Pang Mapha	Mae Hong Son	1.87	3.09	4.28
Mean				2.59	3.38	4.75
Maximum				3.77	4.04	5.58
Minimum				0.94	2.16	3.17

Evaluation of agronomic traits and seed yield potential

In the second year, 24 hemp lines from each accession, which showed seed yield potential in the first year, were selected for evaluation of agronomic traits and seed yield. The experiment was conducted using an augmented randomized complete block design. The method for hemp plantations was identical to the first-year experiment. The morphological and agronomic traits of the hemp plants were collected at the harvesting stage. The following traits were recorded postharvest: Plant height (cm), number of branches per plant, stem diameter (mm), stem weight (g), number of flowers on an inflorescence, inflorescence length (cm), weight of 100 seeds (g), seed weight per plant (g), and seed yield (kg ha^{-1}). Mean comparison, correlation, and path coefficient analysis for seed yield were performed using these morphological and agronomic traits.

Statistical analysis

Data analysis was conducted as follows: (1) The phenotypic correlation of several traits with seed weight per plant was studied. (2) Cluster analysis was performed using squared Euclidean distances, and the average linkage between groups was calculated as the average distance between all pairs of cases within a group. (3) Path coefficient analysis was used to dissect the phenotypic correlation into direct and indirect effect components. This data analysis was performed using R software (R Foundation for Statistical Computing, Vienna, Austria) and the R cluster package (Maechler et al., 2022).

RESULTS

Hemp seed collection

Hemp seeds were collected from 63 planting sites across 11 districts spanning three provinces in northern Thailand. These specific locations were chosen due to their known promotion of hemp cultivation for fiber and textile purposes. Individual seed weights, 100-seed weights, and lengths of the collected seeds are shown in Table 1. The average weight of 100 seeds from the 63 samples was 2.59 g. On average, seed width and length were 4.75 and 3.38 mm, respectively. Seeds collected from Tha Sai Luat, Mae Sot, and Tak had the highest 100-seed weight, while those from Mae Yuw, Mae Sariang, and Mae Hong Son exhibited the lowest. After sowing, only 37 of the accessions developed well in the experimental field. These 37 hemp accessions were used in the study for the evaluation of yield potential.

Cluster analysis

Out of the 37 hemp accessions and two hemp varieties that flourished in the experimental field until harvest, they were categorized into five clusters (Figure 1). Cluster 2 was the most substantial group, comprising 20 hemp accessions, including the hemp varieties RPF1 and RPF2. The hemp accessions in Clusters 5, 4, 3, and 1 included nine, three, five, and two accessions, respectively (Table 2). The average values of height, number of branches, stem diameter, flowers per inflorescence, length, trichome density, seed weight per plant, and 100-seed weight for each cluster are reported in Table 2.

The distinguishing morphological characteristics of hemp in group 1 include a large stem (30.30 mm), long inflorescences (11.25 cm), a high number of flowers in an inflorescence (10.12 flowers), and a substantial seed weight per plant (840.68 g). The characteristics of hemp in Cluster 2 are a small stem size (22.04 mm), short inflorescences (4.23 cm), a low trichome density score (1.76), and a low seed weight per plant (184.38 g). The notable characteristics of hemp in Cluster 3 are a high number of flowers in an inflorescence (11.00 flowers) and a high trichome density score (3.25). The remarkable characteristic of hemp in Cluster 4 is a large stem (32.01 mm) and a high number of branches per plant (29 branches). The standout characteristics of hemp in Cluster 5 are a tall plant height (362.64 cm) and a high seed weight (3.26 g).

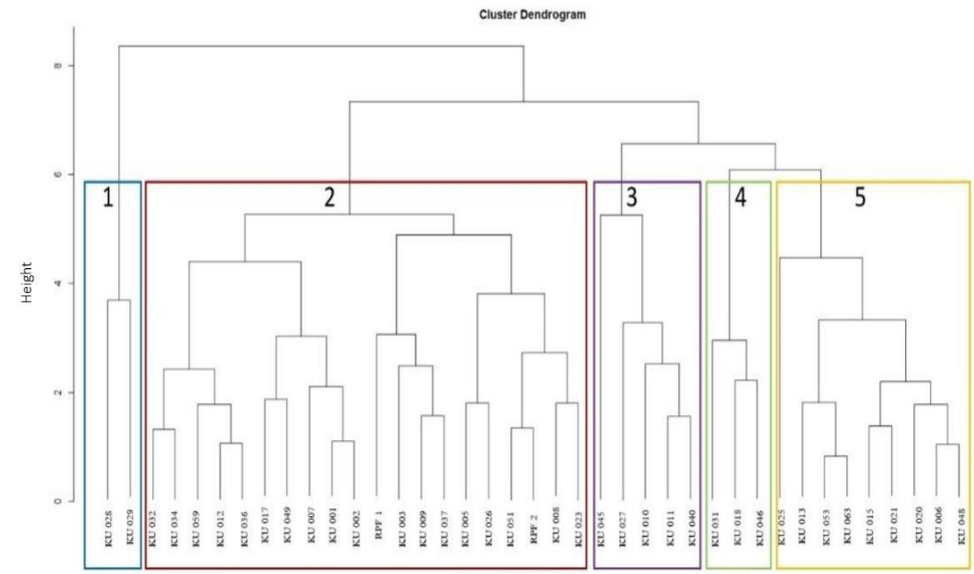

Figure 1. Cluster analysis of hemp accessions and hemp varieties RPF1 and RPF2 based on agronomic characteristics.

Table 2. Characteristics of each hemp group classified by morphological data and yield components. Data presented as mean ± SD.

Clusters	1	2	3	4	5
Number of hemp accessions per cluster	2	20	5	3	9
Height, cm	317.50 ± 10.61	294.04 ± 23.46	299.00 ± 7.42	331.67 ± 20.21	362.64 ± 22.90
Number of branches	21.25 ± 0.42	23.64 ± 3.98	27.55 ± 3.47	29.00 ± 1.50	23.50 ± 2.27
Stem diameter, mm	30.30 ± 3.52	22.04 ± 3.83	21.90 ± 2.52	32.01 ± 4.92	22.07 ± 1.57
Flowers per inflorescence	10.12 ± 1.59	6.68 ± 3.33	11.00 ± 4.43	9.17 ± 3.25	6.52 ± 1.92
Inflorescence length, cm	11.25 ± 0.56	4.23 ± 0.69	4.83 ± 0.33	4.50 ± 0.62	4.50 ± 0.49
Trichome density	2.12 ± 0.53	1.76 ± 0.42	3.25 ± 0.66	1.92 ± 0.14	2.31 ± 0.51
Seed weight per plant, g	840.68 ± 85.31	184.38 ± 119.71	441.83 ± 392.19	484.49 ± 620.87	200.58 ± 65.48
Hundred seeds weight, g	3.15 ± 0.01	2.82 ± 0.53	2.69 ± 0.21	3.22 ± 0.41	3.26 ± 0.24

Correlation analysis

The correlation coefficient analysis for the phenotypic characteristics, including seed weight per plant and 100-seed weight, from the 37 accessions is presented in Table 3. The seed weight per plant showed a significant correlation with inflorescence length (0.50) and stem diameter (0.46). The 100-seed weight exhibited a considerable correlation with plant height (0.44). Additionally, stem diameter was significantly correlated with inflorescence length (0.39).

The second-year data revealed the correlation coefficient analysis of the phenotypic characteristics of the 24 selected hemp lines and control varieties (Table 4). The seed weight per plant had a highly significant correlation with the number of flowers per inflorescence (0.55). However, the 100-seed weight did not show a significant correlation with other traits.

Table 3. Correlation coefficient analysis of morphological and agronomic traits from the 39 hemp accessions in the first year. **, *Significant at 0.01 and 0.05 probability levels, respectively.

Characteristics	Height	Number of branches	Stem diameter	Flowers per inflorescence	Inflorescence length	Trichome density	Hundred seeds weight	Seed yield per plant
Height	1.00							
Number of branches	0.17	1.00						
Stem diameter	0.14	0.08	1.00					
Flowers per inflorescence	-0.11	-0.01	0.27	1.00				
Inflorescence length	0.11	-0.04	0.39*	0.18	1.00			
Trichome density	0.15	0.29	-0.13	0.19	-0.01	1.00		
Hundred seeds weight	0.44**	-0.10	0.22	0.05	0.08	0.03	1.00	
Seed weight per plant	0.10	0.10	0.46**	0.27	0.50**	0.23	0.10	1.00

Table 4. Correlation coefficient analysis of morphological and agronomic traits from the 14 selected hemp lines and checked varieties in the second year. **, *Significant at 0.01 and 0.05 probability levels, respectively.

Characteristics	Height	Branch without seeds	Branch with seeds	Flowers per inflorescence	Stem diameter	Branch length	Inflorescence length	Stem dried weight	Bast dried weight	Hundred seeds weight	Seed yield per plant
Height	1.00										
Branch without seeds	0.40*	1.00									
Branch with seeds	0.10	-0.71**	1.00								
Flowers per inflorescence	-0.06	-0.45**	0.28	1.00							
Stem diameter	0.11	-0.03	0.33	0.22	1.00						
Branch length	0.41*	-0.38*	0.65**	0.36*	0.65**	1.00					
Inflorescence length	0.23	-0.43*	0.59**	0.56**	0.57**	0.85*	1.00				
Stem dried weight	0.69**	0.12	0.31	0.17	0.20	0.44	0.35	1.00			
Bast dried weight	0.80**	0.21	0.28	0.00	0.23	0.49	0.30	0.85**	1.00		
Hundred seeds weight	0.32	0.12	0.00	-0.09	0.04	-0.08	-0.32	0.03	0.18	1.00	
Seed yield per plant	0.34	-0.23	0.30	0.55**	0.11	0.43	0.34	0.35	0.28	0.24	1.00

Path coefficient analysis

The path coefficient analysis results from the first year, which illustrate the direct and indirect influences of various traits on the weight of seeds per plant, are consolidated in Table 5. The traits that had a direct impact on the seed weight per plant were identified as inflorescence length (0.37), stem diameter (0.32), trichome density score (0.25), and 100-seed weight (0.19). Stem diameter and inflorescence length were found to indirectly influence the seed weight per plant. Specifically, the inflorescence length indirectly affected the seed weight per plant through its effect on the stem diameter, with a coefficient of 0.14. Similarly, the stem diameter had an indirect effect on the seed weight per plant through its influence on inflorescence length, with a coefficient of 0.12.

In the second year, Table 5 provides a clear representation of the direct and indirect effects on seed yield. The traits that had a direct and positive influence on hemp seed yield were height (0.73), flowers per inflorescence (0.72), and branch length (0.8). In contrast, stem diameter (-0.79) and inflorescence length (-0.51) were found to have a direct and negative impact on seed yield. The indirect effects, as detailed in Table 6, were also significant, demonstrating the influence of traits such as height, flowers per inflorescence, stem diameter, branch length, and inflorescence length on seed yield.

Table 5. Path coefficient analysis for direct and indirect effect of traits on seed weight per plant in the first year. The numbers written in bold indicate the direct effect.

Characteristics	Height	Number of branches	Stem diameter	Flowers per inflorescence	Inflorescence length	Trichome density	Hundred seeds weight
Height	**-0.09**	0.01	0.04	0.00	0.04	0.04	0.07
Number of branches	-0.01	**0.04**	0.03	0.00	-0.02	0.07	-0.02
Stem diameter	-0.01	0.00	**0.32**	0.00	0.14	-0.03	0.03
Flowers per inflorescence	0.01	0.00	0.09	**0.01**	0.07	0.05	-0.06
Inflorescence length	-0.01	0.00	0.12	0.00	**0.37**	0.00	0.01
Trichome density	-0.01	0.01	-0.04	0.00	0.00	**0.25**	-0.03
Hundred seeds weight	-0.03	0.00	0.05	0.00	0.02	-0.04	**0.19**

Table 6. Path coefficient analysis for direct and indirect effect of traits on seed weight per plant in the second year. The numbers written in bold indicate the direct effect.

Characteristics	Height	Branch without seeds	Branch with seeds	Flowers per inflorescence	Stem diameter	Branch length	Inflorescence length	Stem dried weight	Bast dried weight	Hundred seeds weight
Height	**0.73**	0.03	0.04	-0.04	-0.63	0.32	-0.12	-0.01	-0.08	-0.01
Branch without seeds	0.29	**0.08**	-0.26	-0.33	-0.01	-0.31	0.22	0.00	-0.02	0.00
Branch with seeds	0.07	-0.06	**0.37**	0.20	0.08	0.52	-0.30	0.00	-0.03	0.00
Flowers per inflorescence	-0.04	-0.04	0.10	**0.72**	0.07	-0.28	0.29	-0.28	0.00	0.00
Stem diameter	0.48	0.00	0.12	0.17	**-0.79**	0.60	-0.31	-0.01	-0.07	0.00
Branch length	0.29	-0.03	0.24	0.26	-0.59	**0.80**	-0.43	-0.01	-0.05	0.00
Inflorescence length	0.17	-0.04	0.22	0.40	-0.48	0.68	**-0.51**	-0.01	-0.03	0.01
Stem dried weight	0.51	0.01	0.12	0.12	-0.54	0.35	-0.18	**-0.02**	-0.08	0.00
Bast dried weight	0.59	0.02	0.10	0.00	-0.61	0.39	-0.15	-0.02	**-0.10**	0.00
Hundred seeds weight	0.23	0.01	0.00	-0.07	-0.06	-0.06	0.16	0.00	-0.02	**-0.02**

Evaluation of selected hemp lines for yield potential

The ANOVA revealed that several traits, including height, inflorescence length, branches with seeds, branches without seeds, inflorescences per plant, stem diameter, branch length, inflorescence length, stem dried weight, bast dried weight, and seed weight, were not significantly different between the 24 hemp lines and the control varieties (Table 7). Consequently, these 24 hemp lines were selected for future field trials.

Table 7. Analysis of variances for augmented design on traits of 24 selected hemp lines.

Source of variation	Df	Mean square										
		Height	Branch without seeds	Branch with seeds	Flowers per inflorescence	Stem diameter	Branch length	Inflorescence length	Stem dried weight	Bast dried weight	Hundred seeds weight	Seed weight per plant
Treatment	25	2834.00	20.62	46.23	47.76	0.16	420.80	350.70	6097.00	223.00	12.87	2158.00
Control	1	829.00	4.70	3.71	154.18	0.01	114.00	15.00	1.00	251.70	1.40	8760.00
Test	23	2536.00	21.87	46.77	42.11	0.17	362.30	345.80	6277.00	199.50	13.87	1613.00
Test vs. Control	1	11691.00	7.65	76.49	71.25	0.13	2074.30	798.70	8049.00	732.90	1.29	8100.00
Block	2	657.00	3.56	2.88	40.22	0.00	62.30	239.80	6556.00	82.00	0.06	6399.00
Residuals	2	1578.00	61.93	77.13	147.49	0.01	807.20	532.20	3209.00	96.80	1.56	953.00
CV		9.31	85.54	24.21	70.15	5.31	25.69	31.05	17.65	13.62	4.16	29.53

DISCUSSION

Hemp seeds are in demand by the food and cosmetic industries (Farinon et al., 2020). However, the hemp varieties cultivated in Thailand were primarily developed for fiber production, and thus, no hemp variety has been specifically developed for seed production. This study marks the beginning of hemp breeding for high seed yield production in Thailand.

Clustering germplasm based on valuable traits is a crucial step in the breeding programs of various crops, including hemp (Piluzza et al., 2013; Grigoriev et al., 2020; Pieracci et al., 2021; Amarasinghe et al., 2022; Thongthip et al., 2023). The cluster analysis of the phenotypic traits in our study revealed that the hemp accessions within each cluster did not exhibit any geographical correlation. This suggests that the hemp accessions in northern Thailand may have originated from the same source, including the varieties RPF1 and RPF2. Cluster 2, which consisted of 22 accessions and two varieties collected from eight districts, accounted for 61.54% of the total samples. However, the hemp accessions in Clusters 1, 3, 4, and 5 may have undergone natural selection, resulting in distinctive characteristics compared to their original sources. The agronomic traits of these four clusters differed from those of Cluster 2. Importantly, the hemp accessions in Clusters 1, 3, 4, and 5 exhibited outstanding characteristics for each agronomic trait, making them suitable for breeding programs (Kahraman et al., 2014; Shrestha, 2016).

The correlation analysis conducted in the first and second years revealed different traits significantly associated with the seed weight per plant. In the first year, the analysis indicated that hemp lines with longer inflorescences and larger stem diameters were likely to produce more seeds. Additionally, a positive correlation was observed between inflorescence length and stem diameter, aligning with previous studies (Panda et al., 2020) and underscoring the importance of these traits in achieving high seed yield. However, in the second year, the correlation analysis showed that only the number of flowers per inflorescence was related to the seed weight per plant. This year, hemp lines with large stems and long inflorescences were selected for yield evaluation, but these traits did not significantly affect the seed weight per plant.

Path coefficient analysis has been successful in identifying the direct impact of target traits on other morphological traits, such as waterlogging tolerance, seed yield, and seed protein content (Aman et al., 2020; Khan et al., 2022; Thongthip et al., 2023). The direct effect of traits on seed weight per plant, as shown by path coefficient analysis, differed between the first and second years. In the first year, inflorescence length had the most substantial direct effect on hemp seed weight per plant, followed by stem diameter. In contrast, in the second year, branch length had the highest direct effect on hemp seed weight per plant, followed by height and the number of flowers per inflorescence. In this study, hemp lines with large stems and long inflorescences were selected for the second-year experiment. However, these traits did not have a high effect on seed weight per plant. It was observed that the effect of stem size and inflorescence length on seed weight per plant was higher than that of branch length, height, and the number of flowers per inflorescence, as when the stem size and inflorescence length expressed their effect on seed weight per plant, other traits had a negligible effect.

Based on the seed yield evaluation, the 24 selected hemp lines from the second-year experiment were chosen for the subsequent regional yield trial and used in hemp breeding programs for high seed yields through a single cross-hybridization (Kirana et al., 2024). Furthermore, inflorescence length and large stem diameter, followed by branch length, height, and the number of flowers per inflorescence, emerged as critical traits for selecting hemp genotypes to improve hemp seed yield. These five traits will serve as selection criteria in future hemp breeding programs aimed at high seed yield potential.

CONCLUSIONS

The assessment of hemp accessions indicated that Thai hemp in Cluster 1 exhibited the highest potential for high seed yield production. The correlation analysis identified positive associations among inflorescence length, stem diameter, and seed yield. The path coefficient analysis further confirmed that inflorescence length had the most significant direct effect on seed yield, followed by stem diameter, branch length, height, and the number of flowers per inflorescence. The 24 lines from the second year evaluation were selected for high seed yield potential breeding programs. Then, the morphological traits identified from this study can serve as target traits for selective breeding of hemp, with an emphasis on seed production. The insights gained from this study will guide the selection of hemp accessions in future hemp breeding programs to develop new hemp populations with high seed yield potential.

Author contribution

Conceptualization: T.C., P.T., N.T., B.P., W.P. Methodology: T.C., P.T., N.T. Software: N.T., A.R. Validation: N.T., T.C., S.S., W.P. Formal analysis: N.T., T.C., P.T. Investigation: T.C., B.P., S.S., W.P. Resources: T.C., P.T. Data curation: T.C., N.T. Writing-original draft: N.T., P.T., T.C. Writing-review & editing: T.C. Visualization: T.C., P.T., N.T., B.P., W.P. Supervision: T.C. Project administration: T.C. Funding acquisition: T.C. All co-authors reviewed the final version and approved the manuscript before submission.

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