Clustering of yardlong bean lines and cultivars grown under a drought condition

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Abstract

The effect of drought stress significantly influences yield improvement and declining crop production. Therefore, this experiment aimed to the group and select 25 genotypes of yardlong bean lines and cultivars grown under drought-stress conditions. They were laid out in a randomized complete block design with 3 replications under the water-constrained condition in a rainproof greenhouse. Yield/plant and other 12 horticultural traits were recorded, and multivariate analyses were conducted to assess genetic divergence using Mahalanobis D^2 statistics, and the genotypes were grouped into clusters following Tocher's method. The results showed that yardlong bean lines and cultivars grown under drought were classified into 7 clusters. Clusters I – VII contained 6, 10, 2, 2, 1, 3 and 1 genotype (genotypes), respectively. The inter-cluster distance (D^2) was found to be the maximum between clusters V and VII (52.27), and the same was the minimum between clusters I and IV (11.21). Pod width contributed maximum to the total divergence followed by yield per plant and leaf greenness (SPAD) (22.00, 17.67 and 12.33 %, respectively). Therefore, the clustering of genetic divergence, in selecting lines from crossing between genotypes of clusters V and VII, can achieve high gains of selection and high-yielding lines for planting in drought conditions.

Keywords: asparagus bean, grouping, genetic divergence, drought stress

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Introduction

Yardlong bean [*Vigna unguiculata* (L.) Walp. subsp. *sesquipedalis* (L.) Verdc.] is a member of the Fabaceae family and grown primarily for its crisp, soft green pods, which are eaten either raw or cooked. It is a very nutritious vegetable with 23.50-26.30 % digestible protein in both the pods and the leaves (Ano, & Ubochi, 2008). It is one of Thailand's most important essential vegetables and is grown year-round. According to The Department of Agricultural Extension (2022), the yardlong bean had about 6,437.50 hectares of plantation countrywide and produced about 61,104 tons of crop during the 2021 planting season.

Drought stress, both as a seasonal phenomenon and as part of climate change, is currently the greatest threat to the world's food supply (Budak, Kantar, & Kurtoglu, 2013). The agriculture industries in Thailand have been severely affected by the drought conditions. The data from Thailand's drought survey shows that agricultural areas sustain yearly harm regularly. Agricultural regions affected by drought conditions ranged in size from 10,300-2,197,866 hectares annually from 2002-2017, with an average damage of 333,203 hectares and a cost of 949,039,953 baht (The Secretariat of the House of Representatives, 2019). Drought is the most important abiotic factor that adversely affects growth and crop production, especially in warm and dry areas (Fathi, & Tari, 2016).

Researchers have been working on yardlong bean breeding projects for several years, including gathering cultivars in the provinces of Chonburi and Eastern for planting, testing, and selecting (Kanhong, & Pornsuriya, 2014; Kanhong, Pornsuriya, & Sangueandeegul, 2016). Yardlong bean cultivars were crossed to study the heterogeneity of pod characteristics in their second generations (F_2) (Pornsuriya, & Pornsuriya, 2016). Ketone, Pornsuriya, Ieamkheng, & Mareubodi (2018) and Pornsuriya, Pornsuriya, & Julakasewee (2018) assessed the genetic diversity, heritability, and genetic progress of F_3 yardlong bean lines. Pornsuriya, Pornsuriya, & Chittawanij (2019) experimented with yield and pod properties of yardlong bean lines for line selection. Therefore, lines from the breeding program, including local and commercial cultivars, could be used as germplasm sources for testing and selecting under drought conditions.

The use of multivariate analysis for determining the degree of divergence and for assessing the relative contribution of different characters to the total divergence in self-pollinated crops has been demonstrated by several researchers (Golakia, & Makne, 1992; Bansal, Saini, Rani, & Kaur, 1999; Kohli, & Agarwal, 2002; Narayanankutty, Mili, & Jaikumaran, 2003; Rambabu, Reddy, Kamala, Saidaiah, & Pandravada, 2016; Bhagavati et al., 2018; Thangam, Ramachandrudu, Kumar, & Safeena, 2020). D^2 analysis is one of the most precise multivariate analysis methods used in selecting the parents for hybridization (Hossain et al., 2013). Clustering analysis using Mahalanobis distance grouped the common bean genotypes for drought stress adaptation into four groups showing high and significant inter-cluster distance, suggesting that hybridization between drought-adapted parents from the groups will provide the maximum genetic recombination for drought tolerance in subsequent generations (Darkwa, Ambachew, Mohammed, Asfaw, & Blair, 2016). The success of the hybridization followed by selection depends mainly on the choice of parents showing high genetic diversity for traits of interest from D^2 analysis (Murty, & Arunachalam, 1966). However, there is no sufficient information on the genetic diversity of yardlong bean genotypes using D^2 analysis in Thailand, especially under drought conditions. Breeding is an important technology for the development of crop production systems to support the expansion of the market (Boonlertnirun, Boonlertnirun, Buasanit, & Jompuk, 2018). Therefore, this study aimed to evaluate yardlong bean genotypes grown under the simulated drought condition, estimate genetic divergence in yield and other traits, and group into clusters for future hybridization program utilization.

Methodology

In the experiment, 25-yardlong bean lines and cultivars (Table 1) that were acquired from various genetic resources, including commercial, local, and lines selected from crossbreeding in previous studies (Pornsuriya, Pornsuriya, & Chittawanij, 2019; Pornsuriya, Pornsuriya, Kwun-on, & Chittawanij, 2021) were evaluated. The 25-yardlong bean genotypes were laid out in a randomized complete block design (RCBD) with 3 replications (3 planting blocks) in a rain-protected greenhouse, and there were 2 plants/experimental units. In the simulated drought condition, the soil was watered between 80-100 % field capacity from planting for 30 days; after that, the watering was controlled to keep the soil moisture not exceeding 50 % field capacity throughout harvest. A WET Sensor type WET2 from Delta-T Devices Ltd., UK, was used to measure soil moisture throughout the experiment. Leaf greenness was measured at 49 and 56 days after planting (from observing that plant leaves were affected by drought conditions) using a non-destructive, portable SPAD-502 chlorophyll meter (Minolta Camera Co., Ltd., Japan). The two plants of each experimental unit were collected for observation from each replication for 13 quantitative traits; the average from these two plants was subjected to statistical analysis.

Multivariate analysis was performed utilizing Mahalanobis D^2 statistics, and genotypes were grouped into different clusters following Tocher's method (Singh, & Chaudhary, 2012). The experiment was conducted at The Rajamangala University of Technology Tawan-ok, Chonburi Province, from October to December 2021.

Table 1 List of 25-yardlong bean genotypes and their sources used in this study.

no.	genotype	source	no.	genotype	source
1	No.33P	line from crossbreeding	14	No.19G	line from crossbreeding
2	BangP2	selected line (in the breeding program)	15	No.3P	line from crossbreeding
3	P10T26	line from crossbreeding	16	No.40P	line from crossbreeding
4	BangP1	selected line (in the breeding program)	17	No.22G	line from crossbreeding
5	No.37G	line from crossbreeding	18	No.32P	line from crossbreeding
6	No.30P	line from crossbreeding	19	No.25G	line from crossbreeding
7	LxBP1	line from crossbreeding	20	No.18G	line from crossbreeding
8	BangPP	selected line (in the breeding program)	21	No.10P	line from crossbreeding
9	ChiaS	commercial cultivar	22	No.23G	line from crossbreeding
10	No.33G	line from crossbreeding	23	No.38P	line from crossbreeding
11	P4T8	line from crossbreeding	24	No.20G	line from crossbreeding
12	Nigro	commercial cultivar	25	Nawa3	selected local cultivar
13	Suwan	commercial cultivar			

Results and discussion

Mean performance of genotypes

The mean, standard error (SE) and coefficient of variation (CV) values of each of the 25 genotypes for the 13 characters are shown in (Table 2). The CV is a value that indicates the comparable variance between traits since it is adjusted to the same unit of percentage. Therefore, from (Table 2), the top four characteristics with the greatest variance were number of nodes per plant, fresh plant weight, root length, and yield per plant (32.10, 30.10, 29.40, and 21.50 %, respectively). The least minor variance characteristic was pod width (7.60 %). The findings were consistent with Pornsuriya, & Pornsuriya (2016) who studied the variability in the second generation of the crossbreeding yardlong bean cultivars, which found the slightest variation in pod width traits (9.94 %). Low or high variability of traits implied the scope for improvement of these traits through selection. Regarding yield, the top four genotypes with the highest yield per plant under drought stress were No.33P, P4T8, No.33G and BangP2 (411, 359, 351 and 342 g/plant, respectively).

Table 2 Mean values of the horticultural traits and yield per plant of 25-yardlong bean genotypes.

genotype no.	horticultural traits and yield												
	RL	RW	DA	LG	NB	NN	PVL	FPW	PL	PW	PWE	SP	YP
1	12.30	8.08	32.70	49.80	5.67	19.30	433	395	59.40	0.68	28.40	17.60	411
2	14.30	8.82	32.70	45.00	4.33	16.50	379	275	59.80	0.65	27.20	16.70	342
3	14.20	9.80	32.70	50.50	4.50	17.20	361	255	51.70	0.63	23.70	17.70	318
4	12.80	6.72	33.00	37.50	4.17	15.30	335	263	58.90	0.61	24.50	17.70	305
5	12.70	9.72	33.00	41.80	3.83	15.30	382	252	56.30	0.56	21.30	14.60	295
6	14.30	6.50	33.00	41.20	3.83	19.30	464	305	52.40	0.69	27.70	15.80	283
7	11.80	9.82	33.30	35.20	3.67	15.00	345	247	60.10	0.60	25.90	15.90	302
8	12.00	6.73	33.30	49.10	3.67	15.30	353	238	57.50	0.63	24.70	17.10	291
9	13.80	7.62	33.30	36.70	3.50	17.20	411	270	61.20	0.64	27.50	17.80	270
10	12.30	8.25	34.00	47.20	5.17	18.80	437	357	55.30	0.64	23.80	17.00	351
11	12.50	8.68	33.70	47.00	5.50	19.20	453	367	58.80	0.70	29.60	15.60	359
12	12.30	8.08	34.00	39.30	4.50	15.70	348	328	57.90	0.62	24.30	17.60	293
13	12.50	7.70	34.30	39.60	6.17	20.80	449	370	56.00	0.65	29.60	17.00	319
14	11.30	7.98	34.30	50.10	4.33	15.30	365	330	59.60	0.66	27.10	16.40	298
15	13.70	9.90	34.30	30.90	4.17	15.80	350	330	54.90	0.65	26.10	17.20	298
16	11.70	6.77	31.70	40.70	4.00	16.50	370	333	58.90	0.65	26.50	16.20	274
17	12.00	5.88	32.00	38.00	4.33	13.00	292	275	61.90	0.67	28.30	17.90	281
18	11.00	5.80	32.00	43.80	3.00	15.50	336	283	51.30	0.63	22.50	15.70	254
19	12.50	7.73	32.00	43.80	4.00	14.30	341	232	59.40	0.57	21.40	17.90	294
20	13.00	7.93	32.30	48.10	3.83	15.00	340	232	55.00	0.62	23.60	17.50	231
21	12.20	9.18	31.30	41.20	4.00	15.00	370	310	62.10	0.62	28.00	16.40	208
22	11.80	12.63	31.70	49.70	3.17	13.50	301	237	63.10	0.63	27.10	17.80	262
23	11.80	6.63	31.30	41.60	5.50	16.50	397	347	55.80	0.65	25.20	16.70	313
24	13.20	8.57	31.70	40.00	4.00	16.20	337	270	61.20	0.62	25.10	18.10	256
25	12.80	8.55	32.00	34.90	5.67	17.20	372	388	66.10	0.66	31.00	16.90	320
mean[1/]	12.60	8.16	32.80	42.50	4.34	16.40	373	300	58.20	0.64	26.00	16.90	297
SE[2/]	0.24	0.28	0.30	0.91	0.16	0.38	8.63	10.41	0.72	0.01	0.45	0.23	7.38
CV (%)	16.20	29.40	7.90	18.50	32.10	20.00	20.00	30.10	10.70	7.60	15.00	12.00	21.50

where, RL= Root length (cm), RW= Root weight (g), DA= Days to first anthesis, LG= Leaf greenness (SPAD unit), NB= No. of branches/plant, NN= No. of nodes/plant, PVL= Plant vine length (cm), FPW= Fresh plant weight (g), PL= Pod length (cm), PW= Pod width (cm), PWE= Pod weight (g), SP= No. of seeds/pod, YP= Yield/plant (g)

[1/, 2/] mean, standard error (SE) and coefficient of variation (CV) were calculated from 75 experimental units (25 genotypes × 3 replications).

Grouping of genotypes into different clusters (D^2 analysis)

The Mahalanobis distance (D^2 analysis) finds wide applications in multivariate statistics. It differs from Euclidean distance in that it considers the correlations between variables (Ghorbani, 2019). According to D^2 statistics, the 25 genotypes were divided into 7 clusters with varying numbers of genotypes (Table 3), indicating the presence of a sizable degree of genetic variation in the materials. Among 7 clusters, cluster II was the largest consisting of 10 genotypes, followed by cluster I, VI, III and IV with 6, 3, 2 and 2 genotypes, respectively, where clusters V and VII were presented by only 1 genotype.

Table 3 Distribution of 25-yardlong bean genotypes into 7 clusters.

cluster	number of genotypes	genotype number	name of the genotypes
I	6	1, 10, 11, 2, 14, 8	No.33P, No.33G, P4T8, BP2, No.19G, BPP
II	10	3, 20, 4, 18, 12, 19, 24, 9, 16, 15	P10T26, No.18G, BP1, No.32P, Nigro, No.25G, No.20G, ChiaS, No.40P, No.3P
III	2	5, 7	No.37G, LxBP1
IV	2	6, 23	No.30P, No.38P
V	1	13	Suwan
VI	3	17, 25, 21	No.22G, Nawa3, No.10P
VII	1	22	No.23G

Average intra and inter-cluster distances

The mean intra- and inter-cluster D^2 values are provided in (Table 4). The intra-cluster D^2 values ranged from 0.00 (cluster V and VII) to 10.51 (Cluster VI). The highest intra-cluster D^2 value was recorded by cluster VI (10.51). Clusters V and VII had no intra-cluster distance because each had a single genotype. The inter-cluster D^2 value was maximum (52.27) between cluster V and VII, followed by between clusters IV and VII with a high D^2 value of 41.69 (Table 5), demonstrating more genetic diversity among the genotypes represented by these groups. It would be possible to produce different recombinants by choosing parents for a hybridization program among these diverse clusters. The lowest distance measured between clusters I and IV was 11.21, indicating a strong connection between the genotypes included. Based on D^2 estimates, (Table 5) shows the closest and farthest clusters to each cluster.

Table 4 Intra (diagonal, bold) and an inter-cluster average of D^2 values from 13 traits of 25-yardlong bean genotypes.

clusters	cluster I	cluster II	cluster III	cluster IV	cluster V	cluster VI	cluster VII
cluster I	**7.03**	12.68	15.95	11.21	23.21	19.38	25.94
cluster II		**9.00**	12.79	16.15	26.90	14.99	20.32
cluster III			**5.97**	21.51	34.12	14.15	17.51
cluster IV				**10.29**	17.66	20.44	41.69
cluster V					**0**	24.91	52.27
cluster VI						**10.51**	19.66
cluster VII							**0**

Table 5 The nearest and farthest clusters from each cluster based on D^2 values in 25 yardlong bean genotypes.

clusters	nearest cluster	farthest cluster
cluster I	cluster IV (11.21)	cluster VII (25.94)
cluster II	cluster I (12.68)	cluster V (26.90)
cluster III	cluster II (12.79)	cluster V (34.12)
cluster IV	cluster I (11.21)	cluster VII (41.69)
cluster V	cluster IV (17.66)	cluster VII (52.27)
cluster VI	cluster III (14.15)	cluster V (24.91)
cluster VII	cluster III (17.51)	cluster V (52.27)

The contribution of various traits towards diversity

The contribution of each trait to overall divergence is displayed in (Table 6). Among the traits studied, pod width contributed the maximum to the diversity (22.00 %), followed by yield per plant (17.67 %), leaf greenness (12.33 %), pod length (11.00 %), pod weight (9.67 %), and number of seeds per pod (6.67 %). These 6 traits together contributed 79.34 % towards total divergence. Therefore, to create cultivars that can withstand the effects of drought, these characteristics should be given priority during hybridization and population selection. The vegetative characteristics of root weight, plant vine length, number of branches per plant, number of nodes per plant, root length and fresh plant weight contributed to less diversity, which amounted to 20.66 %. Where days to the first anthesis was no effect on the divergence. Compared to previous studies conducted in normal conditions, for example, Rambabu, Reddy, Kamala, Saidaiah, & Pandravada (2016) reported that pod length ranked first with a maximum contribution of 48.66 %, followed by pod ascorbic acid content (20 %), and Bhagavati et al. (2018) showed that yield/plant exhibited. maximum contribution (38.77 %) followed by ascorbic acid content (28.26 %), and 100 seed weight (11.59 %). The results of this study were inconsistent with those previous studies, possibly due to different genetic sources and environments.

Table 6 Relative contribution of 13 traits towards genetic divergence in 25-yardlong bean genotypes grown under drought condition.

trait abbreviation	traits	no. of times ranked first	contribution (%)
RL	root length (cm)	7	2.33
RW	root weight (g)	15	5.00
DA	days to the first anthesis	0	0.00
CH	leaf greenness (SPAD unit)	37	12.33
NB	no. of branches/plant	11	3.67
NN	no. of nodes/plant	9	3.00
PVL	plant vine length (cm)	13	4.33
FPW	fresh plant weight (g)	7	2.33
PL	pod length (cm)	33	11.00
PW	pod width (cm)	66	22.00
PWE	pod weight (g)	29	9.67
SP	no. of seeds/pod	20	6.67
YP	yield/plant (g)	53	17.67
	total	300	100

Cluster means of characteristics

The mean values of 7 clusters are presented in (Table 7). Cluster I recorded the highest mean value for yield per plant, indicating that the segregating progeny from intra-cluster hybridization would produce segregants with the highest yield per plant compared to the other intra-cluster segregation progeny. The genotypes from cluster IV earned the highest cluster mean values for root length and pod width. The first position with respect to days to anthesis, number of branches per plant, number of nodes per plant, plant vine length, fresh plant weight and pod weight was established in cluster V, which had only 1 genotype.

Genotypes in cluster VI had the highest mean value for pod length. Cluster VII, which had only 1 genotype, earned the highest cluster mean values for root weight, leaf greenness and number of seeds per pod. Cluster II and III did not possess the first ranking for any characteristic.

Knowledge of the genetic diversity of a plant population helps a plant breeder in the selection of desirable parents for a breeding program by grouping them into clusters according to the genetic distance. Lowered genetic advance typically results from crossing closely related genotypes, (Porch et al., 2013); consequently, future breeding programs using the investigated genotypes should be based on genetic analysis of various traits and hybridization carried out among clusters rather than within clusters. Crosses involving parents from the most divergent clusters are predicted to exhibit the highest levels of genetic recombination and genetic architecture variation (Arunachalam, 1981). Similar works had been done to select suitable parents for breeding programs, for example, Rambabu, Reddy, Kamala, Saidaiah, & Pandravada (2016); Bhagavati et al. (2018); Thangam, Ramachandrudu, Kumar, & Safeena (2020).

Table 7 Mean values of 7 clusters by Tocher’s method for the horticultural traits and yield per plant in 25 genotypes of yardlong bean (the highest value of each character is in bold number).

traits	clusters							
	Abb.	I	II	III	IV	V	VI	VII
root length (cm)	RL	12.47	12.82	12.25	13.08	12.50	12.33	11.83
root weight (g)	RW	8.09	7.89	9.77	6.57	7.70	7.87	12.63
days to anthesis	DA	33.44	32.70	33.17	32.17	34.33	31.78	31.67
leaf greenness (SPAD unit)	CH	48.03	41.13	38.48	41.38	39.55	38.02	49.70
no. of branches/plant	NB	4.78	3.97	3.75	4.67	6.17	4.67	3.17
no. of nodes/plant	NN	17.40	15.90	15.20	17.90	20.80	15.10	13.50
plant vine length (cm)	PVL	403	353	363	430	449	344	301
fresh plant weight (g)	FPW	327	280	249	326	370	324	237
pod length (cm)	PL	58.40	57.00	58.20	54.10	56.00	63.40	63.10
pod width (cm)	PW	0.66	0.62	0.58	0.67	0.65	0.65	0.63
pod weight (g)	PWE	26.80	24.50	23.60	26.50	29.60	29.10	27.10
no. of seeds/pod	SP	16.70	17.30	15.30	16.30	17.00	17.10	17.80
yield/plant (g/plant)	YP	342	279	299	298	319	269	262

Conclusion

The 25-yardlong bean genotypes evaluated under simulated drought conditions revealed that four genotypes with the highest yield per plant were No.33P, P4T8, No.33G and BangP2. Genetic divergence assessed using Mahalanobis' D^2 statistics revealed that the 25 genotypes could be grouped into 7 clusters. The highest inter-cluster distance was observed between cluster V and VII followed by clusters IV and VII. Among the 13 traits studied, pod width contributed the most to the diversity, followed by yield per plant, leaf greenness, pod length, pod weight, and number of seeds per pod. Cluster I showed the highest mean value for yield per plant, followed by cluster V.

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