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Efficient test sites for multi-environment evaluation of sugarcane genotypes in Thailand

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Abstract

Multi-environment trials (METs) of crop genotypes are costly and require efficient test sites for cost effectiveness. This study aimed to identify efficient test sites for METs of sugarcane (Saccharum spp.) genotypes in Thailand, utilizing data from 10 sugarcane genotypes conducted at nine locations covering different sugarcane growing regions of the country for two crop-classes. Cluster analysis and the genotype plus genotype \times environment (GGE) biplot method were used to group these sites into five subsets, based on their similarity in genotypic responses of cane and sugar yields of the planted crop and the first ration crop. The results showed a fair agreement between the two methods, but inconsistent results were obtained from groupings that were based on different yield traits and crop-classes. Locations appearing more consistent in certain groups were chosen as the representatives of the respective groups to constitute the set of efficient test sites. Cluster analysis and the GGE biplot, however, identified different sets of test sites that were equally effective in retaining the $G \times L$ interaction and the performance ranking of the test genotypes as the original nine test sites. The selected locations by cluster analysis which included Nakhon Ratchasima, Ratchaburi, Kamphaeng Phet, Tha Phra, Khon Kaen and Udon Thani are preferred because of their wider geographical distribution. Four sites could thus be omitted, which would substantially reduce the costs and time and greatly improve the efficiency of the METs of sugarcane genotypes in Thailand.

Keywords: Multi-environment trials (METs); Environment grouping; GGE biplot; Cluster analysis; Breeding line evaluation.

Introduction

An increasing market demand for sugar and increased use of ethanol as a renewable alternative energy have created a strong need to increase sugarcane (Saccharum officinarum L.) production worldwide. In Thailand, sugarcane is a major upland crop being grown in different regions of the country, occupying an area of 977,956 ha in 2010, with a total production of 68.81 million tons and an average fresh cane yield of 70.36 t ha⁻¹ (FAOSTAT 2012). The country is a major world sugar exporter and the use of ethanol has increased rapidly in recent years as a result of government promotion policy. Considerable efforts have been made to improve production of sugarcane in the country, and major emphasis has been allocated to varietal improvement. Currently, there are sugarcane breeding programs of both government agencies and private companies. Just like in other crops, a major task of these sugarcane breeding programs is the multi-environment trials (METs) of crop breeding lines to identify superior genotypes. METs are needed because of differential responses of genotypes to different environments (genotype \times environment (G×E) interaction) and consequently causing a change in performance rankings of the test genotypes in different environments (Annicchiarico, 2002; Kang, 2002; Baenziger et al., 2006).

Conducting METs, however, is laborious, time consuming and costly. More inputs are required for sugarcane because plot size for METs is large, crop duration is long and includes both the plant and ratoon crop. Because of resource constraints, it has not been possible for a sugarcane breeding program in Thailand to evaluate advanced breeding lines over the entire range of environmental conditions in different regions of the country. To overcome this problem, a Thailand program for coordinated METs of elite sugarcane genotypes was recently initiated. The program combines efforts and resources of the participating government agencies and private companies in conducting the trials at the respective locations within the areas of their responsibility. This program has made it possible to test elite sugarcane genotypes over 8-12 locations in different parts of the country each year. However, the overall time and resources spent on this program are quite large, posing a large burden for continuing the program in the long run. Improving the cost effectiveness of these trials is, thus, strongly needed.

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It has been well recognized that only the crossover type of $G \times E$ interaction is associated with significant genotypic rank change and, thus, has significant implication on the multi-environment evaluation of crop breeding lines (Annicchiarico, 2002; Crossa et al., 2002; Kang, 2002). Performance ranking of the test genotypes would be the same if two or more test sites have no or non-crossover $G \times E$ interaction and testing would only be needed at one of these sites (Annicchiarico, 2002; Kang, 2002). A set of complementary test sites that adequately sample the environments of interest with minimal duplication is required for an efficient testing of crop breeding lines (Roozeboom et al., 2008). The use of inappropriate test sites will not only lower the effectiveness of breeding line evaluation, but is also a waste of valuable time and resources.

Appropriate test sites for breeding line evaluation have been investigated in several crops utilizing available METs data that covered a wide range of geographical regions. These included spring wheat (Triticum aestivum L.) (Navabi et al., 2006), winter wheat (Triticum aestivum L.) (Collaku et al., 2002; Yan and Tinker, 2005; Roozeboom et al., 2008), rice (Oryza sativa) (Fan et al., 2001), soybean (Glycine max (L.) Merr.) (Yan and Rajcan, 2002), sorghum (Sorghum bicolor) (Mgonja et al., 2008) and lentil (Lens culinaris Medik.) (Naser et al., 2012). The general approach is to first group the test sites based on their similarity in genotypic responses, and then select a representative site from each group. Several procedures have been used in grouping the test environments. Clustering techniques using squared Euclidean distance as the dissimilarity measure and incremental sum of squares or Ward's strategy (Ward, 1963) as the clustering strategy are the methodologies that have been used extensively in subdividing the test locations (Collaku et al., 2002; Russel et al., 2003). Lately, the genotype and genotype \times environment interaction (GGE) biplot method (Yan et al., 2000) has become popular, including its use in environmental grouping (Navabi et al., 2006; Roozeboom et al., 2008) and evaluating similarities between test environments (Dehghani et al., 2006; Setimela et al., 2010; Zhe et al., 2010; Ramburan et al., 2012). As efficient test sites are important to improve the effectiveness and reduce the cost of sugarcane variety evaluation and this has not been investigated in Thailand, this study was conducted to identify efficient test sites for METs of sugarcane genotypes in Thailand.

Materials and Methods

Multi-environment trials

This study utilized data from the Thailand coordinated METs of elite sugarcane lines conducted during the years 2005-2008. The conduct of the trials was a cooperative effort among government agencies and private companies involved in the sugar industry in Thailand, with financial support from the National Science and Technology Development Agency (NSTDA). The trials were conducted at nine locations in sugarcane growing areas in different regions of the country (Table 1).

The genotypes evaluated were 10 elite sugarcane lines from breeding programs of various organizations in Thailand. They were Kps94-13, TBy20-0535 and TBy20-0154 from Kasetsart University (KU), Suphanburi 80 and Uthong 8 from the Suphan Buri Agricultural Research and Development Center, Khon Kaen 3 from the Khon Kaen Field Crops Research Center, MPT96-273 and MPT96-392 from the Mitr Phol Innovation and Research Center and K84-200 and K88-92 from the Cane and Sugar Industry Promotion Center Region 1, the Office of the Cane and Sugar Board.

At each location, a randomized complete block design with four replications was used. Plot size was 4 rows, 10 m long, with a spacing of 1.3 m between rows and 0.5 m between plants (52 m²). Planting was done manually with double seed setts (2-3 buds sett⁻¹). The trials in clay soil were irrigated after planting, sprayed with pre-emergence herbicide for weed control, and supplied with supplementary irrigation one to two times at tillering and elongation stages, while the trials in sandy soil were not irrigated. Fertilizer application for the plant crop was based on soil analysis for each location. Weeding was done as necessary. Crop durations for the planted crop varied from 10-13 months. The ratoon crop was fertilized at the same rate as the plant crop, and weeding was done as necessary. The duration for the ratoon crop was 12-13 months.

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No.CodeNamecoordinatesSoilconditionseasonsdatePlantRatoonPlantRatoonL1NMANakhon $15'34N$ TypicRainfedLR8 Dec 052.2 Jan 0711 Feb 08992960L2KKN IDonhun, $16'20N$ TypicRainfedLR8 Dec 052.2 Jan 0711 Feb 08992960L3RBRRatchasima $102'53'E$ PaleustultsRainfedLR24 Nov 0515 Jan 0729 Jan 081,2011,093L3RMNNakhon $15'33'N$ $0xyaquicIrrigatedER28 Apr 0625 Mar 0721 Mar 089591,044L4NSNSawan102'57EHaplustalfsIrrigatedER18 May 0620 Jan 073 Mar 081,2211,320L4NSNSawan102'07EHaplustalfsIrrigatedER18 May 0620 Jan 073 Mar 081,2211,324L6KPTKhamphaeng16'28NTypicIrrigatedER28 Jan 0620 Mar 0721 Jan 081,043L6KPTKhamphaeng16'22NTypicIrrigatedER23 May 065 April 0760 Mar 071,032L6KN2Tha Plara,16'22NTypicIrrigatedER23 May 065 April 0760 Mar 071,093L6KKN2Tha Plara,16'22NTypicIrrigatedER23 May 065 April 07<$		Loc	ation	Geooranhical		Water	Planting	Plantino	Harvesti	ing date	Rainfal	l (mm)
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L ₉ UDN Udon Thani 17°25'N Typic Irrigated D 2 Mar 06 12 Feb 07 10 Feb 08 1,324 1,25	L_8	KRI	Kanchanaburi	14°01'N 99°38'E	Oxyaquic Haplustalfs	Irrigated	ER	15 May 06	15 Mar 07	13 Mar 08	1,286	1,155
	L_9	NDN	Udon Thani	17°25'N 102°35'E	Typic Kandiustults	Irrigated	D	2 Mar 06	12 Feb 07	10 Feb 08	1,324	1,255

Table 1. Descriptions of the test locations in the present study.

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Data collection

Prior to planting, soil samples were collected from all nine experimental fields to a depth of 0-30 cm using a hand augur. The soil samples were analyzed for organic matter (OM), available phosphorus (P), exchangeable potassium (K) and pH. Rain and temperature data for each site were obtained from the nearest meteorological station.

Data were recorded from the center two rows of each plot on percent germination at one to two months after planting, number of stools at two months after planting, number of tillers per stool at three months after planting and disease and pest damages at three months after planting. At final harvest, the center two rows with 10 m long (26 m^2) in each plot were harvested. The number of millable stalks was counted and cut at ground level, and stalk fresh weight per plot was recorded. A sub-sample of ten stalks per plot was randomly taken to determine agronomic traits and yield components, i.e., stalk length, stalk weight and stalk diameter. Juice was extracted by applying 19.3 MPa for 30 s using a cane sample press (Model SP-9, J&L Honiron, Louisiana, USA) for quality traits analysis. Brix was determined on the juice using an automatic temperature compensated (20 °C) refractometer (Model ATR-SW, Schmidt and Haensch, Berlin, Germany). For the determination of pol, 2.5 g of lead acetate was thoroughly mixed with the juice, which was then filtered through a Whatman No. 91 filter paper and the filtrate was passed through a Polarimeter (Polartronic NIR W₂, Schmidt and Haensch, Berlin, Germany). The fresh and dry weights of the remaining stalk material were determined for calculation of fiber content. Dry weight of sugar yield per plot was then calculated based on adjusted CCS value as: CCS=3/2 P (1-(F+5)/100)- $\frac{1}{2}$ B (1-(F+3)/100); where P=Pol at 20 °C, B=Brix at 20 °C and F=Fiber (%); Sugar yield=(CCS×Cane Yield)/100.

Data analysis

Cane and sugar yields from the multi-environment trials were first statistically analyzed for each environment by a conventional analysis of variance procedure. Error variances were tested for their homogeneity using the Bartlett's test as outlined by Gomez and Gomez (1984). The test indicated homogeneity of error variances, thus, combined analysis of variance for the trials over nine environments was performed based on the following model:

 $Y_{ijkl} = \mu + L_i + R_{ij} + G_k + (LG)_{ik} + \varepsilon_{ijk} + C_l + (LC)_{il} + (GC)_{kl} + (LGC)_{ikl} + \delta_{ijkl}$

Where:

 Y_{ijkl} is the yield in location *i* in replicate *j* within location *i* of genotype *k* and crop class *l*,

 μ is the overall mean,

 L_i is the effect of location *i*,

 \mathbf{R}_{ij} is the effect of replication *j* within location *i*,

 G_k is the effect of genotype k,

 $(LG)_{ik}$ is the interaction between location *i* with genotype *k*,

 ε_{ijk} is the error associated with main-plot unit in location *i*, replication *j* within location *i* and genotype *k*,

 C_l is the effect of crop class l,

 $(LC)_{il}$ is the interaction between location *i* with crop class *l*,

 $(GC)_{kl}$ is the interaction between genotype k with crop class l,

 $(LGC)_{ikl}$ is the interaction between location *i* with genotype *k* and crop class *l*,

 δ_{ijkl} is the error associated with location *i*, replicate *j* within location *i*, genotype *k* and crop class *l*.

The Statistix 8 software program (Analytical Software, Tallahassee, FL) was used for data analysis.

Two methods were used in location grouping, i.e., cluster analysis (Collaku et al., 2002) and the GGE biplot method (Yan, 2001). Cluster analysis was used to group the nine test sites into subsets based on their similarity in the genotypic responses for cane yield and sugar yield. A hierarchical cluster analysis with Ward's method algorithm (Ward, 1963) was used to group the test locations. Test site groupings were done separately for the planted crop, the ratoon crop and the average of the two crop-classes. All analyses were done using SAS proc CLUSTER and TREE (SAS institute 1996). The grouping was truncated when the R^2 among groups exceeded 85%. The sites that showed consistency in their grouping between the plant crop and the ratoon crop for both cane yield and sugar yield were selected to represent the respective environmental groups. Their testing efficiency was then determined by the relative magnitude of G×E interaction and the relative performance of the test lines compared to those of the trials over all nine original sites.

Location grouping with the GGE biplot method was performed using the GGE biplot software (Yan, 2001). The GGE biplot was constructed from the

first two components (PC_1 and PC_2) that were derived from exposing environment-centered yield data to singular value decomposition (SVD), based on the following formula:

 $Y_{ij} - \beta_j = \lambda_1 \xi i_1 \eta j_1 + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij},$

Where:

 Y_{ij} is the yield of genotype *i* in environment *j*,

 β_j is the average yield over all genotypes in environment *j*,

 λ_1 and λ_2 are the singular values for PC₁ and PC₂, respectively,

 ξ_{i1} and ξ_{i2} are the eigenvectors of genotype *i* for PC₁ and PC₂, respectively,

 η_{j1} and η_{j2} are the eigenvectors of environment *j* for PC₁ and PC₂, respectively,

 ε_{ij} is the residual of the model associated with the genotype *i* in environment *j*.

Since an "ideal" test location for genotype evaluation should discriminate the test genotypes and be representative of the target-environments (Yan et al., 2007), both the discriminating power and the representativeness were used as the criteria in location grouping. For the GGE biplot, the length of a location vector, i.e., the line that connects the biplot origin and the marker of the location, indicates the discriminating power, i.e., the longer is the vector the higher is the discriminating power of the location. The angle between a location vector and the average environment coordinate (AEC) abscissa indicates the representativeness, i.e., the smaller is the angle the higher is the representativeness of the location. The length of the location-vector and the angle between location-vectors were thus used as the criteria for location grouping. Similar to the cluster analysis method, test site groupings were done separately for the planted crop, the ratoon crop and the average of the two crop classes based on cane yield and sugar yield. The sites that showed consistency in their grouping between the plant crop and the ration crop were selected to represent the respective environmental groups. Their testing efficiency was also determined by the relative magnitude of G×E interaction and the relative performances of the test lines compared to those of the trials over all nine original sites. The results from the two methods were also compared.

Results and Discussion

Diversity of test locations and genotypes

The nine test locations for the coordinated METs of sugarcane lines in Thailand were intentionally selected to cover the geographical regions and environmental conditions representing the different production areas of sugarcane in the country. They are located in all regions that sugarcane is grown (Figure 1). These locations extend from 13° 44' N to 17° 25' N latitude and 99° 27' E to 102° 50' E longitude. The individual test locations also differed in soil type, planting season, irrigation management, planting date and harvesting date. Rainfalls during the experimental period at these test locations ranged from 951 to 1,324 mm for the plant crop and from 960 to 1,609 mm for the first ration crop (Table 1). The diversity in environmental conditions and management practices resulted in a great variation in crop productivity among locations. Average cane yield at these locations varied from 71.2 to 127.1 t ha⁻¹ for the plant crop, from 40.5 to 113.1 t ha⁻¹ for the first ration crop and from 61.1 to 106.9 t ha⁻¹ for the average of the two crop-classes. Average sugar yield ranged from 8.67 to 17.79, 6.28 to 15.91 and 7.53 to 16.04 t ha⁻¹ for the plant crop, the first ration crop and the average of the two crop-classes, respectively (Table 2).

		Can	e yield (t l	na ⁻¹)	Suga	r yield (t l	na ⁻¹)
Code	Location	Plant	Ratoon	Moon	Plant	Ratoon	Moon
		crop	crop	Ivicali	crop	crop	Wiean
L ₁	Nakhon Ratchasima	81.74	40.51	61.13	13.02	6.28	9.65
L_2	Donhun, Khon Kaen	98.13	70.02	84.08	12.88	10.03	11.46
L_3	Ratchaburi	71.18	52.82	62.00	8.67	6.40	7.53
L_4	Nakhon Sawan	75.33	76.73	76.03	10.48	9.81	10.15
L_5	Chaiyaphum	127.06	79.76	103.41	17.79	10.15	13.97
L_6	Khamphaeng Phet	79.53	75.42	77.47	10.11	10.58	10.35
L_7	Tha Phra, Khon Kaen	90.94	76.36	83.65	13.97	10.89	12.43
L_8	Kanchanaburi	92.57	113.12	102.85	11.17	15.91	13.54
L ₉	Udon Thani	113.04	100.73	106.89	17.62	14.46	16.04
	Mean	92.17	76.16	84.17	12.86	10.54	11.68

Table 2. Means for cane and sugar yields for the different test locations.



Figure 1. Geographical positions of the nine test locations for METs of elite sugarcane genotypes conducted during 2005-2008.

The ten sugarcane genotypes used in present study included eight elite breeding lines and two released cultivars. They differed considerably in growth pattern, maturity and cane yield level. Average cane yields for these sugarcane genotypes over all locations ranged from 72.2 to 115.3 t ha⁻¹ for the plant crop, from 57.48 to 95.02 t ha⁻¹ for the first ratoon crop and from 64.85 to 105.14 t ha⁻¹ for the average of the two crop-classes. Average sugar yields ranged from 10.36 to 15.87 t ha⁻¹ for the plant crop, from 8.07 to 13.16 t ha⁻¹ for the first ratoon crop and from 9.22 to 13.93 t ha⁻¹ for the average of the two crop-classes (Table 3).

Combined analysis of variance also indicated large variations among locations and among genotypes for both cane yield and sugar yield. Variations among locations were the largest source of variation for both cane yield and sugar yield, with the location sum of squares being 27.5 and 26.9% of total sum of squares for cane yield and sugar yield, respectively. Variations among genotypes were second, with the sum of squares for genotype being 14.1 and 13.0% of total sum of squares for cane yield and sugar yield, respectively (Table 4). A wide coverage of environments in the target area and diverse genotypes are basic requirements for an effective determination of test sites. Data from across years are also recommended for test site classification (Russell et al., 2003; Yang et al., 2005). The diversities of the test locations and the test genotypes described above and the coverage of two crop-classes which occurred in different years should sufficiently fulfill the basic requirements for the determination of efficient test sites.

Table 3. Means and ranks for cane yield and sugar yield of the test genotypes that were obtained from the trials over the nine original test sites.

		Plant	crop	Ratoon	crop	Aver	age
Code	Genotype	Mean (t ha ⁻¹)	Rank	Mean (t ha ⁻¹)	Rank	Mean (t ha ⁻¹)	Rank
Cane yie	eld (t ha ⁻¹)						
G ₁	Kps94-13	99.08	4	83.21	3	91.15	4
G_2	TBy20-0535	72.22	10	57.48	10	64.85	10
G_3	TBy20-0154	83.66	7	65.86	9	74.76	8
G_4	Suphanburi 80	91.09	6	79.50	4	85.30	6
G_5	Uthong 8	81.72	8	73.84	7	77.78	7
G ₆	KhonKaen 3	99.95	3	85.65	2	92.80	3
G_7	MPT96-273	95.18	5	75.91	6	85.54	5
G_8	MPT96-392	109.26	2	78.51	5	93.89	2
G_9	K84-200	74.27	9	66.65	8	70.46	9
G_{10}	K88-92	115.27	1	95.02	1	105.14	1
LDS 0.0	95, df 347	12.64		13.07		9.44	
Sugar yi	eld (t ha ⁻¹)						
G ₁	Kps94-13	14.95	2	11.94	3	13.45	2
G_2	TBy20-0535	10.36	10	8.07	10	9.22	10
G ₃	TBy20-0154	10.70	8	8.22	9	9.46	9
G_4	Suphanburi 80	11.73	6	10.30	6	11.02	6
G_5	Uthong 8	11.36	7	10.23	7	10.80	7
G_6	KhonKaen 3	14.71	3	13.16	1	13.93	1
G_7	MPT96-273	14.18	4	10.64	5	12.41	5
G_8	MPT96-392	15.87	1	10.64	4	13.26	4
G_9	K84-200	10.57	9	9.41	8	9.99	8
G ₁₀	K88-92	14.15	5	12.38	2	13.26	3
LDS 0.0	05, df 347	2.03		1.90		1.45	

		SS (% of T	$SS)^{a}$				SS (%	of TSS) ^a of f	ive sets of s	elected tes	t sites			
Source	0	riginal 9 tes	t sites		Se	t 1	Se	st 2	Set	3	Se	t 4	Se	t 5
SOULCE	η£	Cane	Sugar	- 10	Cane	Sugar	Cane	Sugar	Cane	Sugar	Cane	Sugar	Cane	Sugar
	Ħ	yield	yield	Ð	yield	yield	yield	yield	yield	yield	yield	yield	yield	yield
Location (L)	8	27.45**	26.88^{**}	4	31.66^{**}	32.27**	32.80^{**}	33.77**	30.23**	34.44**	30.85**	35.91***	19.43^{**}	11.93^{**}
Rep/L (R/L)	27	6.94	5.55	15	5.62	4.75	7.91	6.20	7.02	5.44	10.10	7.24	7.02	6.65
Genotype (G)	6	14.09^{**}	12.95^{**}	6	12.05^{**}	9.53^{**}	8.25^{**}	7.51**	14.40^{**}	10.98^{**}	10.02^{**}	8.15**	12.49^{**}	12.37^{**}
GxL	72	7.10^{**}	7.02^{**}	36	6.13^{**}	6.44^{**}	$5.03^{\rm ns}$	$5.00^{ m ns}$	7.42**	6.81^{**}	$5.82^{\rm ns}$	5.59^{ns}	7.99**	9.41^{**}
Pool error (a)	243	15.41	14.33	135	13.71	13.21	17.03	15.02	13.31	12.88	17.75	15.14	15.66	14.53
Crop class (C)	1	6.68^{**}	6.27**	1	18.02^{**}	18.21^{**}	12.89^{**}	13.41^{**}	13.86^{**}	13.93^{**}	9.00^{**}	9.47**	19.31^{**}	20.13^{**}
L×C	×	10.33^{**}	13.73^{**}	4	3.64^{**}	4.31^{**}	5.94^{**}	7.94^{**}	2.98^{**}	2.68^{**}	4.26^{**}	5.75**	6.82^{**}	10.96^{**}
G×C	6	1.07^{**}	1.67^{**}	6	$0.53^{\rm ns}$	1.22^{**}	$0.54^{\rm ns}$	1.36^{**}	1.85^{**}	2.01^{**}	1.75^{*}	2.09^{**}	1.21^{**}	1.67^{**}
G×L×C	72	3.12^{*}	3.20^*	36	2.67^{**}	2.62^{ns}	2.74^{*}	2.61^*	2.39^{*}	$2.61^{\rm ns}$	$2.68^{\rm ns}$	2.73^{ns}	3.14^{**}	3.44^{*}
Pool error (b)	270	7.81	8.41	150	5.97	7.45	6.86	7.16	6.53	8.22	7.77	7.94	6.93	8.91
CV (%)		16.79	19.11		16.58	20.19	17.87	20.07	16.15	19.90	17.58	19.74	16.15	18.65
ns *** non-sign	ificant	and signifi	cant at P≤0	0.05 and	10.01, resp	sectively.								
^a Sum of square	s exnre	ssed as ner	reent of tots	al sum o	of squares									
make to mare	·	we we have	1001 TO 111001	· · · · · · · · · · · · · · · · · · ·	on and and to	_								

Table 4. Combined analysis of variance for cane yield and sugar yield of 10 elite sugarcane genotypes evaluated under the original nine test sites and under five sets of five selected test sites.

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Test sites grouping and identification of efficient test sites

Test site grouping was intended to group the test sites that have no or non-significant $G \times E$ interaction together, and then select a representative site from each group for actual testing. It was anticipated that the representative sites would still maintain the total $G \times E$ interaction pertaining to the target region which is required for performance evaluation of crop genotypes. In the present study, grouping of the test locations was done by cluster analysis (Collaku et al., 2002) and by the GGE biplot method (Yan, 2001) based on cane yield and sugar yield of the planted crop, the ratoon crop and the average of the two crop-classes.

Grouping by cluster analysis was truncated at five groups, with R^2 values among groups for cane yield of the plant crop, the ratoon crop and the average of the two crop-classes being 0.87, 0.91 and 0.90, respectively (Figure 2). The grouping based on sugar yield gave R^2 values among groups of 0.89, 0.93 and 0.87 for the plant crop, the first ratoon crop and the average of the two crop-classes, respectively (Figure 3). The high R^2 values obtained indicated that truncation at five groups was appropriate as most of the G×L interaction was captured in location grouping.

The results of location grouping showed that the locations within the individual groups that were obtained for different crop-classes, i.e., planted crop or ratoon crop, based on different yield traits, i.e., cane yield or sugar yield, were rather inconsistent (Table 5). For examples, L_3 , L_4 and L_6 were grouped together as Group 2 based on cane yield of the planted crop, but only L_3 was classified as Group 2 based on cane yield of the ratoon crop. Likewise, L_6 and L_8 were in Group 3 on the basis of sugar yield of the planted crop, but four locations (L_4 , L_5 , L_6 and L_7) were classified as Group 3 when grouping was based on sugar yield of the ratoon crop. However, there were some locations that more consistently fell into certain groups across yield traits and crop-classes.

The inconsistency of location groupings between the planted crop and the ratoon crop for both cane and sugar yield could be explained by the highly significant crop-class \times location (C×L) interactions (P<0.01) which accounted for 10.33 and 13.73% of the total variations in cane yield and sugar yield, respectively (Table 4). This indicated that the relative yield levels of the different locations for the planted crop were different from those of the ratoon crop. Sugarcane is a perennial crop which is harvested for yield in about 11-12 months for both the planted crop and the ratoon crop. Thus, the two crop-classes were under environmental conditions of different years. In additions, the G×C interactions were also highly significant (P<0.01) indicating that the relative performances of the genotypes differed in the different crop-classes. From the perspective of the G and GE effects, the differences in genotypic performances between the planted crop and the ratoon crop were confounded with the season or year effect, and thus, complicating the across years analysis of genotype or location performances. Yang et al. (2005) also found little or no repeatability of test site groupings across years in their study on green pea in North America. Results of location grouping based on cane yield also differed somewhat from those based on sugar yield. This could also be expected as sugar yield is a function of cane yield and CCS and these two traits are not related and are sometimes negatively correlated.

The results also revealed that location grouping was not related to geographical regions as locations within a group were found in different geographical regions and locations within a geographical region could also be classified as different groups. For example, L₄ and L₆ which are in the Central Region were grouped together in Group 4 with L_5 and L_7 which are in the Northeast, when grouping was based on cane yield of the ratoon crop (Table 5 and Figure 1). Likewise, L1, L2, L5 and L9 which are in the Northeast were classified as different groups when grouping was based on cane yield of the planted crop. Locations that are geographically close together could also be classified as different groups because of their differences in soil type and field condition. For example, L_7 and L_9 are close together geographically (Figure 1) but have different soil types (typic haplustalfs for L_7 and typic kandiustults for L_9) (Table 1); they were classified as different groups on every basis (Table 5). Locations that have the same soil type and are geographically close could also be classified as different groups, e.g., L₁ and L₂. These results are in line with previous studies which showed the complex and unpredictable nature of G×E interactions that were influenced by several factors some of which could randomly differ in type and magnitude from year to year (Kimbeng et al., 2002; Navabi et al., 2006).

une ratioon crop.										
Docid for manine.		Groupir	ng by cluster	analysis			Grouping t	oy GGE biplo	t method	
Dasis Ior grouping	Group 1 ^a	Group 2 ^a	Group 3 ^a	Group 4 ^a	Group 5 ^a	Group 1 ^a	Group 2 ^a	Group 3 ^a	Group 4 ^a	Group 5 ^a
Cane yield, plant crop	L1	L_3, L_4, L_6	L_2, L_7, L_8	Ls	L ₉	L1	L_2	L_4, L_7, L_8	L_5, L_9	L_3, L_6
Cane yield, ratoon crop	L_1	L_3	L_2	L_4, L_5, L_7	L_8, L_9	L_{l}	L_4	${ m L}_2,{ m L}_3,{ m L}_5,\ { m L}_7,{ m L}_9$	L_8	L_6
Cane yield, average of two crop-classes	L_1, L_3	L_2	${ m L}_4$	L_6, L_7	L_5 , L_8 , L_9	L_1	L_2	L_4, L_9	$\mathrm{L}_3,\mathrm{L}_5,\\mathrm{L}_4$	L_{6}
Sugar yield, plant crop	L_1, L_2, L_7	L_3	L_6, L_8	L_4	L_5, L_9	L_1	L_2	L_4, L_7, L_8	L_3, L_5, L_9	L_{6}
Sugar yield, ratoon crop	L_1, L_3	L_2	L_4, L_5, L_7	L_8	L_9	L_{l}	L_2, L_3, L_8	L_9	L_4, L_6	L_5, L_7
Sugar yield, average of two crop classes	L_1	L_3	L_2, L_4, L_6	${ m L}_5, { m L}_8, { m L}_7$	L_9	L_1	L_2, L_4, L_9	L_3, L_7	L_5, L_8	L_6
^a See location descriptions	in Table 1.									

Table 5. List of test locations in the individual groups that were derived from cluster analysis based on cane yield and sugar yield of the plant crop and the ratio crop.



Figure 2. Clustering of the nine test locations for METs of elite sugarcane genotypes in Thailand based on cane yield of the plant crop (a), of the first ration crop (b) and of the average of two crop-classes (c). The dashed line is the cutoff point for location grouping. L_1 =Nakhon Ratchasima, L_2 =Donhun, Khon Kaen, L_3 =Ratchaburi, L_4 =Nakhon Sawan, L_5 =Chaiyaphum, L_6 =Khamphaeng Phet, L_7 =Tha Phra, Khon Kaen, L_8 =Kanchanaburi, L_9 =Udon Thanee.



Figure 3. Clustering of the nine test locations for METs of elite sugarcane genotypes in Thailand based on sugar yield of the plant crop (a), of the first ration crop (b) and of the average of two crop-classes (c). The dashed line is the cut off point for location grouping. L₁=Nakhon Ratchasima, L₂=Donhun, Khon Kaen, L₃=Ratchaburi, L₄=Nakhon Sawan, L₅=Chaiyaphum, L₆=Khamphaeng Phet, L₇=Tha Phra, Khon Kaen, L₈=Kanchanaburi, L₉=Udon Thanee.

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With the inconsistency of test site grouping, selecting the sites that are more consistent in location grouping as the representatives of their respective groups was taken as a strategy to identify efficient test sites. The reason for taking this strategy was that the selected site would consistently differ from each others in their interactions with the test genotypes under changing environments over years and thus should maximize the G×E interaction which is required for cultivar evaluation. In this regard, L_1 and L₉ stood out to be consistent in being in Group 1 and Group 5, respectively, for all basis of location grouping by cluster analysis (Table 5), thus, they were chosen as the representative sites for these two groups. L_3 was also more consistent in being in Group 2 than other locations, thus, it was selected as the representative site for this group. The locations within Groups 3 and 4, however, varied depending on the basis from which they were derived. For Group 3, L_2 was consistent in being in this group on the basis of cane yields of the planted and the ratoon crops and average sugar yield of the two crop-classes, but L_6 was consistent on the basis of sugar yield of the individual crop-classes (Table 5). These two locations were initially selected as the candidates to represent Group 3. Likewise, L₅ was consistent in being in Group 4 on the basis of cane yields of the planted and the ration crops and average sugar yield of the two crop-classes, but L_7 was more consistent on the basis of cane yield of the ratoon crop, and average cane and sugar yields of the two crop-classes. Thus, L_5 and L_7 were initially selected as the candidates for the representative site of Group 4. With two candidate sites for two groups, four sets of selected sites were possible. These includes L_1 , L_2 , L_3 , L_5 and L_9 for Set 1, L_1 , L_3 , L_5 , L_6 and L_9 for Set 2, L_1 , L_2 , L_3 , L_7 and L_9 for Set 3 and L_1 , L_3 , L_6 , L_7 and L_9 for Set 4.

The results of the GGE biplot method indicated that, for cane yield, the GGE biplot model accounted for 78.9, 73.8 and 83.2% of the total variations for the plant crop, the ratoon crop and the average of the two crop-classes, respectively (Figure 4), while for sugar yield, the model explained 79.5, 77.8 and 85.3% of the total variations for the plant crop, the ratoon crop and the average of the two crop-classes, respectively (Figure 5). These percentages were sufficiently high for the results of the GGE biplot to be meaningful. Except for L₁, the maximum angles covering all other locations for the individual yield traits and crop-classes were less than 90° (Figures 4 and 5), indicating that all the test locations except L₁ were positively correlated (Yan and Rajcan, 2002). L₁ was far apart from other locations in location-angle, thus, it would be less correlated with other locations. This test location had rainfall less than 1,000 mm throughout the crop duration and less rainfall than other sites for both the plant crop and the first ratoon

crop (Table 1). To be comparable with cluster analysis, the locations were sub-divided into five groups and grouping was done separately for the plant crop, the ratoon crop and the average of the two crop-classes based on cane yield and sugar yield, as done for cluster analysis.



Figure 4. Location groupings by the GGE biplot based on cane yield of the plant crop (a), of the first ration crop (b) and of the average of two crop-classes (c). L_1 =Nakhon Ratchasima, L_2 =Donhun, Khon Kaen, L_3 =Ratchaburi, L_4 =Nakhon Sawan, L_5 =Chaiyaphum, L_6 =Khamphaeng Phet, L_7 =Tha Phra, Khon Kaen, L_8 =Kanchanaburi, L_9 =Udon Thanee.



Figure 5. Location groupings by the GGE biplot based on sugar yield of the plant crop (a), of the first ration crop (b) and of the average of two crop-classes (c). L_1 =Nakhon Ratchasima, L_2 =Donhun, Khon Kaen, L_3 =Ratchaburi, L_4 =Nakhon Sawan, L_5 =Chaiyaphum, L_6 =Khamphaeng Phet, L_7 =Tha Phra, Khon Kaen, L_8 =Kanchanaburi, L_9 =Udon Thanee.

The results of location groupings by the GGE biplot method partly agreed with those obtained from cluster analysis, but many locations were grouped differently, considering that the group numbers by the two methods might not exactly correspond to each other as they were arbitrarily assigned (Table 5). For examples, both cluster analysis and the GGE biplot method identified L_1 as the consistent location being distinctly different from the others (designated as Group 1 by both methods). On the contrary, L₉ consistently fell into one group (Group 5) on every basis by cluster analysis, but it was assigned to different groups (Group 3 and Group 4) by the GGE biplot depending on the yield trait and crop-class. Similarly, on the basis of cane yield of the ration crop, L₄, L₅, L₆ and L₇ were grouped together (Group 4) by cluster analysis but L_5 was grouped together with L_7 , L_2 , L_3 and L_9 (Group 3) by the GGE biplot. The disparities between the results of the two methods were expected because cluster analysis grouped the locations based on the G×L interaction (Collaku et al., 2002) while both the genotypic effect (G) and the $G \times L$ interaction were the basis for location grouping with the GGE biplot method (Yan, 2001).

Similar to cluster analysis, the results of location grouping by the GGE biplot method showed no pattern of association between locations within a geographical region, as shown by locations that are geographically close together being classified as different groups. For example, L_2 and L_7 are in Khon Kaen province in the Northeast but were classified as Group 2 and Group 3, respectively (Figure 1 and Table 5). Locations that have the same soil types but are far apart geographically could also be classified as different groups. For example, L_2 in the Northeast and L_3 in the Central Region (Figure 1) have the same soil types (typic haplustalfs) (Table 1), but were mostly classified as different groups (Table 5).

Inconsistency of the locations within the individual groups for the different yield traits and crop-classes was also obtained from location grouping by the GGE biplot method (Table 5). As done for the cluster analysis method, the location that most consistently fell into a particular group across yield traits and crop-classes was chosen as the representative site for that group. On this basis, L_1 , L_2 , L_7 , L_5 , and L_6 were selected as the representative test sites for Groups 1, 2, 3, 4 and 5, respectively. These sites were somewhat different from those obtained from cluster analysis. This set of sites was designated as Set 5 for subsequent comparisons.

Efficiency in breeding lines evaluation of different sets of selected test sites

The efficiency in breeding line evaluation of the identified test sites was determined by the relative magnitude of genotype \times location (G×L) interaction and the relative performances of the tested lines compared to those obtained from the trials over all the original nine sites. To be efficient, the sites selected as representatives of the individual groups should retain most of the G × location-groups interaction, and thus, should capture most of the total G×L interaction of the nine original locations. The results showed that the relative magnitudes of the G×L interaction obtained from the five sets of selected sites differed to some extent (Table 4). Set 5 derived from the GGE biplot was the best in capturing the highest percentage of G×L interaction for both cane yield and sugar yield. The G×L sum of squares (SS) for this set amounted to 7.99 and 9.41% of total SS for cane yield and sugar yield, respectively, which were even slightly higher than those obtained from the trials over the original nine sites (7.10% and 7.02% of total SS for cane yield and sugar yield, respectively) (Table 4). Set 3 was second but being the best among the four sets that were derived from cluster analysis. This set of test site had G×L SS of 7.42 and 6.81% of total SS for cane yield and sugar yield, respectively, which were comparable to those of the original nine sites. These two sets of test sites appeared to be able to maintain the relative magnitude of the G×L interaction of the original nine sites. However, Set 5 had higher shares of variations due to crop-class and C×L interaction but had lower variations among locations and among genotypes than those of the original nine sites, indicating less coverage of environmental differences and less power to differentiate the test genotypes. Set 3, on the other hand, was able to maintain more or less the same shares of variations for all sources as those of the original nine sites (Table 4), thus, Set 3 has an advantage over Set 5 on this matter.

The magnitude of the G×L interaction alone may not be a good indicator of the efficiency of the test sites, because only the crossover type of G×L interaction is important in test site determination. Performance ranking would be more important in genotypic evaluation. To see whether the selected sets of test sites would give the same results in relative performances of the test genotypes as those obtained from the original nine sites, means and ranks for cane yield and sugar yield of the test genotypes from the five sets of selected test sites were compared with those of the original nine sites. The results showed that, although the absolute values for both cane yield and sugar yield from the reduced sets were slightly different from those from the full set, the ranks of the test genotypes were similar. Examples are shown in Table 6 for Set 3 and Set 5 compared to those of the original nine sites in Table 3. Rank correlations between means of the genotypes over the original nine sites and those of the five individual sets of selected sites were very high for both cane yield and sugar yield of the plant crop and the ration crop, with the rank correlations ranging from 0.88-0.99 (P<0.01) (data not shown). These results indicated that location grouping was effective in capturing the essential part of the G×E interaction that would influence the change in ranking of the test genotypes. Set 5 test sites gave very high rank correlations of the genotypic performance with the original nine sites for both cane yield and sugar yield of the plant crop and the ratoon crop, with the rank correlation values for cane yield of the plant crop, the ratoon crop and the average of the two crop-classes being 0.99, 0.96 and 0.96 (P<0.01), respectively, while the values being 0.89, 0.96 and 0.98 (P<0.01), respectively for sugar yield. Rank correlations of genotypic means of Set 3 with those of the original nine sites were also very high, being 0.99, 0.95 and 0.99 (P<0.01) for cane yield and 0.95, 0.92 and 0.98 (P<0.01) for sugar yield of the plant crop, the ration crop and the average of the two crop-classes, respectively (data not shown). Although there were some switching in rankings of some genotypes for both cane yield and sugar yield, both Set 3 and Set 5 test sites identified the same genotypes in the top 50% (five out of ten) for average sugar yield over the two crop-classes, i.e., G_1 , G_6 , G_7 , G_8 and G_{10} , as did the trials over the original nine sites (Table 6). Both sets also identified four out of five genotypes in the top 50% for average cane yield over the two crop-classes that were identified by the original nine sites, i.e., G_1 , G_6 , G_8 and G_{10} . In addition, both Set 3 and Set 5 identified the same top genotypes for average cane yield (G_{10}) and average sugar yield over the two crop-classes (G_6) as did the original nine sites. Thus, both Set 3 and Set 5 test sites were equally effective in the evaluation of the relative performances of the sugarcane genotypes. In fact, the other three sets, i.e., Set 1, Set 2 and Set 4, also identified the same superior genotypes as described above (data not shown), but they captured less $G \times L$ interaction than the original nine sites (Table 4) and were thus considered slightly inferior to Set 3 and Set 5.

Table 6. Means over two crop-classes and ranks for cane yield and sugar yield of the test genotypes that were obtained from the trials over the original nine test sites and the five selected test sites in Set 3 and Set 5.

Constra	Original nine	e sites	Set 3 selected	test site ^a	Set 5 selected t	est sites ^b
Genotype	Mean (t ha ⁻¹)	Rank	Mean (t ha ⁻¹)	Rank	Mean (t ha ⁻¹)	Rank
Cane yield	$(t ha^{-1})$					
G ₁	99.27	5	85.51	4	89.50	3
G_2	77.41	10	58.69	10	67.31	10
G_3	84.61	8	74.11	8	74.65	7
G_4	99.21	6	81.64	5	81.12	5
G ₅	86.74	7	76.71	7	72.49	8
G_6	102.85	2	87.37	3	89.05	4
G_7	101.28	3	77.18	6	80.39	6
G_8	99.37	4	91.17	2	93.70	2
G_9	79.34	9	63.64	9	69.01	9
G ₁₀	118.45	1	99.46	1	102.24	1
Sugar yield	l (t ha ⁻¹)					
G ₁	14.80	4	13.14	2	13.22	3
G_2	11.03	9	8.62	10	9.72	9
G_3	10.40	10	10.20	8	9.62	10
G_4	12.94	6	10.92	6	11.11	6
G_5	12.16	7	10.91	7	10.21	7
G_6	15.78	1	13.48	1	13.57	1
G_7	14.92	3	11.52	5	11.86	5
G_8	14.29	5	13.00	3	13.18	4
G_9	11.40	8	9.44	9	9.95	8
G_{10}	14.94	2	12.99	4	13.28	2

^a Set 3 includes L_1 , L_2 , L_3 , L_7 and L_9 .

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^b Set 5 includes L_1 , L_2 , L_5 , L_6 and L_7 . See location descriptions in Table 1.

Overall, both Set 3 and Set 5 were as effective as the original nine sites in performance evaluation of sugarcane genotypes, and were considered at par in being the efficient test sites. However, Set 3 has an advantage over Set 5 in the ability to maintain the same shares of variations for all sources as those of the original nine sites, and the locations in Set 3 (L_1 , L_3 , L_6 , L_7 and L_9) are more widely distributed geographically than the locations in Set 5 (L_1 , L_2 , L_5 , L_6 and L_7) (Figure 1). Considering that the participating agencies in conducting the Coordinated METs of elite sugarcane genotypes in Thailand have areas of responsibility in different parts of the country, Set 3 would be preferred for practical purposes. In fact, Set 3 and Set 5 have three locations in common, i.e., L_1 , L_6 and L_7 . L_1 and L_6 were generally on the opposite sides of the GGE biplot while L_7 was in the middle (Figures 4 and 5) indicating that

they represented three distinct types of environments. L_1 had low rainfall and sandy soil (typic paleustults) (Table 1), thus, represented the drought prone area. L_6 had relatively high rainfall and clay soil (aquic haplustalfs) with irrigation and early-rainy season planting, thus, represented a more favorable environment for the clay soil and early-rainy season planting system. L_7 also had moderately high rainfall and sandy soil (typic paleustults) with late-rainy season planting, thus, represented a typical environment for the sandy soil and late-rainy season planting system. These three locations could be considered as the core sites for the METs, with the additional two locations being supplementary sites for a good coverage of the range of environments in different sugarcane production areas of the country.

Conclusions

Five locations that included L_1 (Nakhon Ratchasima), L_3 (Ratchaburi), L_6 (Kamphaeng Phet), L_7 (Tha Phra, Khon Kaen) and L_9 (Udon Thani) were identified as the set of efficient test sites for METs of sugarcane genotypes in Thailand. These five test sites were equally effective in performance evaluation of sugarcane genotypes as the original nine test sites. With this finding, four sites could be omitted from the trials, which would substantially reduce the costs and time for conducting the METs of sugarcane genotypes in Thailand. Such an improvement would make it possible to continue the Coordinated METs of Elite Sugarcane Genotypes Program in Thailand in the long run with limited funds.

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