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# Research Article Identification of Effective Test Sites Represented to Mega-Environment for Sugarcane Breeding Programs in Thailand

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# **Abstract**

**Background and Objective:** Crop genotypes must go through costly multi-environment experiments (METs), which need suitable test sites to enhance cost effectiveness. This research aims to establish an effective and representative ecological zoning division to enable practical sugarcane cultivar choices. **Materials and Methods:** The experiment made use of a Randomized Complete Block Design (RCBD) with four replications. The studies were conducted at 22 test sites throughout the nation's sugarcane-growing areas. Eight sugarcane genotypes, comprising five genotypes and three commercial checks, were evaluated in this research. Hierarchical clustering or cluster analysis was done to determine the differences between these locations. **Results:** Based on optimal genotype ranking, Kps 01-12 (G8) and KK3 (G6) were the best performers, having a high mean yield and outstanding stability in the examined environment. Test site grouping was developed with the intention of constructing test sites with negligible G×E interactions to choose a representative site from each group for actual testing. Due to location grouping's ability to capture the majority of G×L interaction, truncation at 12 groups was recommended by the high R-square achieved. **Conclusion:** By reducing the 22 locations to 12 sites, which would considerably save costs and time, the METs of sugarcane genotypes in Thailand may be improved.

 $\textbf{Key words:} \ \ \textbf{Multi-environment trials, G} \times \textbf{E interaction, hierarchical clustering, stability, variety selection}$ 

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Data Availability: All relevant data are within the paper and its supporting information files.

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## **INTRODUCTION**

One of the main crops, sugarcane (Saccharum spp.), provides around 75% of the world's sugar. It is also used as a source of bioenergy. In tropical and subtropical climates, where dryness is widespread in some of those locations, this crop is widely planted<sup>1</sup>. Because the crop is grown in the late rainy season and matures through the dry season without extra watering, early dryness frequently affects sugarcane production in Thailand. In addition to soil type and fertility, crop output is also affected by other elements including weeds, diseases and insect pests, which raises the price. The cane yield is influenced by a variety of variables in diverse geographical places where the environment is usually very changeable, especially in newly developed areas. The influences of biotic (insect and disease) and abiotic (soil, management and climate) elements together have a significant impact on cane production<sup>2</sup>. Productivity may be maintained by choosing sugarcane varieties that are appropriate for certain locations. Variety in sugarcane is another important factor that greatly affects production.

Before the release of a varietal, genotypes with either wide or particular adaptability are identified in a multi-location trial on many clones in various environments<sup>3</sup>. In a previous study, only clay soil in the early and late rainy seasons, with or without irrigation, was used in Thailand to examine the stability and adaptation of sugarcane types for yield<sup>4</sup>. The most effective test sites were found in the Northeast, Central Plain and North, according to the Klomsa-Ard *et al.*<sup>4</sup> KK3 was the genotype that performed best across all conditions. Variations in rainfall and rain distribution cause sugarcane genotypes to react to seasons differently. As a result, additional research is required, especially in areas where sugarcane production is developing, to cover newer, more diverse growth circumstances, such as season, irrigation and soil types, in various parts of the nation.

A collection of complementary test sites that are sufficiently sampled from the areas of interest with little duplication are necessary for the efficient evaluation of genotypes<sup>5</sup>. The effectiveness of evaluation programs can be increased and time and costs can be saved, by genotyping evaluation at the right test sites. Since the correct test sites ensure the success of selection programs, the test sites must be accurate representations of the target settings<sup>6</sup>. The representative locations must be able to offer details on the performance of the genotypes similar to that of the target environments. Breeders must sample the suitable environmental circumstances likely to be experienced by the

target settings under which the anticipated genotypes will finally be grown due to the existence of genotypes by environment interaction (GEI)<sup>7</sup>. As a result, optimal test sites should be more representative of the entire locality and be able to distinguish between genotypes in terms of the genotypic main effect8. Yan and Tinker9 emphasized that effective test sites for choosing typically adapted genotypes are those that are both discriminating and representative. Zhao and Li<sup>10</sup> contend that geographical location and adaptability are factors in how sugarcane is affected by climate change. Nine test sites that represent three sections of the desired environment were previously used to evaluate experimental genotypes of sugarcane in Thailand. Currently, Thailand's sugarcane industry has extended to the country's east and west, which are areas with different soil types like sandy and clayey and relatively little rainfall, although some areas can manage irrigation. Planting locations for sugarcane are dispersed across many regions and new places need to be studied to determine the representative test sites for sugarcane genotypes evaluation in the future.

A selection and testing program's characteristics would depend on the scope and nature of GEI in various environments<sup>11</sup>. Numerous factors, such as rainfall, soil texture and soil fertility, limit sugarcane productivity in Thailand. These factors, which vary by region, would influence selection decisions. As a result, it's critical to reassess and choose the finest test sites that most accurately reflect the current target population of habitats. The sugar sector will continue to expand with an emphasis on the management of rain-fed crops under changing climatic circumstances that increase rainfall uncertainty and raise the risk of drought on yields<sup>12</sup>. While genotypes with specific adaptation to environments and soil types should be employed under irrigated conditions, generally adapted cultivars should be grown under rain-fed situations<sup>13</sup>. The newly recommended varieties with resistance to climatic variations that are derived from improved cane genotypes could also have significant unintended consequences for local ecosystems. Finding effective test sites for sugarcane genotypes in Thailand was one of the goals of this study.

# **MATERIALS AND METHODS**

**Study area:** In different regions of the country from 2014 to 2017, four sugarcane cropping systems (late rainy season and early rainy season, late rainy season (LI) and early rainy season (EI) based on irrigated conditions and late rainy season (LR) and early rainy season (ER) based on rain-fed conditions) were

Table 1: Soil physic-chemical properties and weather data at the experimental site management of agricultural practices at 22 test sites

Locations	Conditions	Region	Soil texture	Sand (%)	Silt (%)	Clay (%)	Organic matter	рН	Rain fall (mm)
L <sub>1</sub> , NSN1	LR	Central	Clay loam	25.76	35.16	39.08	1.90	5.89	1,353
L <sub>2</sub> , KPT1	LR	North	Loam	41.06	37.92	21.02	0.35	6.02	1,360
L <sub>3</sub> , SKW1	LR	East	Clay loam	27.86	35.3	36.84	1.99	6.59	1,049
L <sub>4</sub> , NMA1	LR	Northeast	Loamy sand	76.03	18.47	5.50	0.92	5.43	1,029
L <sub>5</sub> , NMA2	LR	Northeast	Loamy sand	81.22	13.07	5.71	2.10	7.07	1,307
L <sub>6</sub> , RYG1	LR	East	Sand	89.68	9.31	1.01	0.62	5.58	1,314
L <sub>7</sub> , UDN1	LR	Northeast	Sandy loam	70.01	19.71	10.28	1.11	5.91	1,317
L <sub>8</sub> , RYG2	LR	East	Sandy loam	77.84	10.99	11.17	0.20	5.97	1,307
L <sub>9</sub> , NSN2	LI	Central	Loam	29.79	48.95	21.26	1.71	6.15	1,444
L <sub>10</sub> , KRI1	LI	Central	Sandy loam	60.06	23.69	16.25	0.96	7.31	1,135
L <sub>11</sub> , SPB1	LI	Central	Clay loam	28.98	30.65	40.37	1.07	7.06	971
L <sub>12</sub> , SKW2	LI	East	Clay	21.73	27.64	50.63	1.79	5.90	1,095
L <sub>13</sub> , KKN1	LI	Northeast	Loamy sand	79.35	16.13	4.52	0.47	6.08	1,267
L <sub>14</sub> , NMA3	LR	Northeast	Sandy loam	54.89	29.4	15.71	1.25	7.03	1,059
L <sub>15</sub> , KRI2	ER	Central	Loamy sand	79.91	17.06	3.03	1.06	5.65	971
L <sub>16</sub> , SPB2	ER	Central	Sandy loam	66.14	26.72	7.14	0.69	7.30	1,033
L <sub>17</sub> , PKN1	ER	Central	Sandy loam	59.57	31.1	9.33	0.40	5.43	685
L <sub>18</sub> , NSN3	ER	Central	Clay	11.42	31.02	57.66	1.26	7.03	1,132
L <sub>19</sub> , NSN4	El	Central	Silty clay loam	11.90	55.96	32.14	2.14	6.23	1,444
L <sub>20</sub> , NSN5	EI	Central	Sandy loam	67.67	22.73	9.60	1.20	6.94	1,341
L <sub>21</sub> , SPB3	El	Central	Loam	36.89	41.59	21.52	1.29	6.27	971
L <sub>22</sub> , PCK1	El	North	Clay	10.01	38.00	60.99	3.80	6.06	1,178

Tak Fa, Nakhon Sawan (NSN1), Bueng Samakkhi, Khamphaeng Phet (KPT1), Watthana Nakhon, Sa Kaeo (SKW1), Khon Buri, Nakhon Ratchasima (NMA1), Pimai, Nakhon Ratchasima (NMA2), Meuang, Rayong (RYG1), Kumphawapi, Udon Thani (UDN1), Ban Khai, Rayong (RYG2), Kao Liao, Nakhon Sawan (NSN2), Bo Phloi, Kanchanaburi (KRI1), U Thong, Suphan Buri (SPB1), Watthana Nakhon, Sa Kaeo (SKW2), Meuang, Khon Kaen (KKN1), Khong, Nakhon Ratchasima (NMA3), Lao Khwan, Kanchanaburi (KRI2), Dan Chang, Suphan Buri (SPB2), Pranburi, Prachuap Khiri Khan (PKN1), Tak Fa, Nakhon Sawan (NSN3), Kao Liao, Nakhon Sawan (NSN4), Krok Phra, Nakhon Sawan (NSN5), U Thong, Suphan Buri (SPB3), Pho Thale, Phichit (PCK1), late rainy rainfed condition (LR), late rainy irrigated condition (LI), early rainy rainfed condition (ER) and early rainy irrigated condition (EI)

used in multi-environment experiments (METs) across the 22 test locations in areas where sugarcane is grown (Table 1).

**Experimental design:** A randomized complete block design (RCBD) with four replications was used in the experiments. The 48 m<sup>2</sup> plot with 4 rows, each 8 m long, 1.5 m between rows and 0.5 m between plants. Cut as a cane sett with three buds per each part of the stalk and two cane sett were used for planting in each stool. Eight sugarcane genotypes including five genotypes (KK06-501 (G1), CSB06-2-15 (G2), CSB06-2-21 (G3), TBy27-1385 (G4), TBy28-0348 (G5)) and three commercial checks, KK3 (G6), LK92-11 (G7) and Kps01-12 (G8) were evaluated in this study. These genotypes were derived from different sugarcane breeding programs i.e., Khon Kaen Field Crops Research Center, Department of Agriculture Thailand (DOA), the Office of the Cane and Sugar Board (OSCB) and Kasetsart University (Kamphaeng Saen Campus).

**Field experiments:** The planting dates were in the late rainy season (October to January) and the early rainy season (April to June) for each condition. However, the late rainy season (LI) and early rainy season (EI) based on irrigated conditions were provided with supplemental irrigation 1-2 times at the tillering and elongation phase. The late rainy

season (LR) and early rainy season (ER) based on rain-fed conditions were largely rain-fed basis. Each plot received an immediate application of a base fertilizer (15-15-15 of N-P<sub>2</sub>O<sub>5</sub>-KO<sub>2</sub>) at a rate of 312.5 kg ha<sup>-1</sup> and a second application of the same fertilizer at the same rate (312.5 kg ha<sup>-1</sup>) four months later. The ratoon crop received two applications of chemical fertilizer (15-15-15 of N-P<sub>2</sub>O<sub>5</sub>-KO<sub>2</sub>) at a rate of 468.78 kg ha<sup>-1</sup>, 4 months after the planted crop was harvested. Weed control, insect damage and disease infestation were avoided and eliminated for best results. The planted crop's duration ranged from 10 to 14 months, whereas the ratoon crop's duration was 12 months.

**Parameters observed:** The data collection involves the physical and chemical properties of soil. Soil samples (each experimental location has 10 sample points that were bulked) were collected from all 22 experimental fields prior to planting at a depth of 0-30 cm using a hand augur. Physical soil analysis, soil texture (sand, silt and clay%) and chemical soil analysis (the laboratory standard methodology was followed to measure the available P, exchangeable K, organic matter (OM) and pH of the soil) was measured. At the final harvest, the traits were recorded from the two middle rows at harvest by counting the number of millable canes

(stalks/plots). The stalk number will be counted and cut at ground level, with stalk fresh weighing per plot to assess cane yield (kg/plot). The data collection of quantitative information randomly selected 8 millable canes from 2 middle rows that were weighed from each subplot tie and labeled the plot number and variety clearly sent to the laboratory to assess juice will be extracted from the 8-stalk subsample for measurements of percent Brix, polarity and fiber, then calculated the commercial cane sugar (CCS).

**Statistical analysis:** Prior to performing the analysis of variance, Bartlett's test was used to determine the homogeneity of the three parameters, ton cane per hectare (TCH) and ton sugar per hectare (TSH). A combined ANOVA of 22 locations for the plant and the first ratoon cane was used to perform an analysis of variance on the measured data using a randomized complete block design. The least significant difference (LSD) test at the 0.05 probability level was used to compare genotypes. Using the Statistics 8 program, all statistical analyses were performed. Their testing effectiveness was then assessed based on the relative strength of the G×E interaction and the test lines' performance in comparison to the trials conducted across all 22 original sites. The JMP software version 16.0 was used to do a cluster analysis utilizing hierarchical clustering. The genotypic responses for ton cane per hectare (TCH) and ton sugar per hectare (TSH) are used to distinguish the different groups based on how similar they are. To represent the various environmental groupings, the sites with consistent TCH and TSH values in their grouping between the average of plant cane, ration cane and the averaged two crop classes were chosen.

#### **RESULTS**

## Best test environment (location) for sugarcane genotypes:

The 22 test locations for the coordinated METs of sugarcane genotypes in Thailand were chosen with the goal of representing the various sugarcane production regions of the nation's geographical distribution and environmental circumstances. They can be found in any area that grows sugarcane. Additionally, the various test locations varied in terms of planting season, irrigation schedule, soil type and soil characteristics.

For the typical plant cane and first ration cane, the amount of rainfall during the experimental period at various test locations ranged from 971 to 1, 444 mm (Table 1). There was a significant difference in crop productivity between places due to the diversity of environmental factors

and management techniques. The mean ton cane per hectare (TCH) for these locations ranged from 43.16 to 166.40 tons for plant cane, from 49.60 to 123.06 tons for the first ratoon cane and from 46.38 to 133.69 tons for the average of two crop classes. For the plant crop, the first ratoon crop and the average of two crop classes, respectively, the mean sugar ton per hectare (TSH) at these locations ranged from 4.88 to 19.16, 5.99 to 17.87 and 5.45 to 17.99 tons per hectare (Table 2).

Five elite breeding lines and three commercial cultivars were among the eight sugarcane genotypes employed in the current investigation. In terms of growth, maturity and cane output, they varied greatly. The range of average cane yields for these sugarcane genotypes across all sites was 73.89 to 103.43 tons per hectare for plant cane, 61.81 to 86.14 tons per hectare for first ratoon cane and 68.40 to 94.78 tons per hectare for the average of two crop classes. For the plant crop, the first ratoon crop and the average of two crop classes, these sugarcane genotypes' average sugar yields across all locations varied from 7.90 to 13.35, 7.51 to 12.10 and 7.71 to 12.73 tons per hectare, respectively (Table 3).

**Test sites evaluate cane and sugar yield for environmental grouping:** The goal of test-environment evaluation is to find test environments that efficiently discover superior genotypes for a mega-environment. To choose a representative site from each group for real testing, test site grouping was designed to combine test sites with insignificant  $G \times E$  interactions together. The overall  $G \times E$  interaction relevant to the target region, which is necessary for the performance assessment of crop genotypes, was expected to remain at the representative locations. Based on the cane yield and sugar yield of plant cane and first ratoon cane, cluster analysis was used in the current study to generate a list of test locations for each group (Table 4).

The sugarcane trials were grouped in the cluster analysis using JMP's hierarchical clustering, which also included measures of the test contexts' desirability, representativeness and power of discriminating. The data were standardized and analyzed at 22 test sites for METs of elite sugarcane genotypes in Thailand based on the discrimination power of the test environment on cane and sugar production of different genotypes. The environments that were investigated can be divided into 6, 8, 10 and 12 groups, with an R-square of 0.9101, 0.9526, 0.9725 and 0.9814, respectively between the groups. The dashed line indicates the location grouping cutoff point for the following six groups: Group 1 includes L<sub>1</sub>, L<sub>4</sub>, L<sub>9</sub>, Group 2: L<sub>19</sub>, Group 3: L<sub>2</sub>, L<sub>18</sub>, L<sub>5</sub>, L<sub>13</sub>, L<sub>3</sub>, L<sub>7</sub>, L<sub>10</sub>, L<sub>20</sub>, Group 4: L<sub>11</sub>, L<sub>21</sub>, Group 5: L<sub>6</sub>, L<sub>12</sub>, L<sub>16</sub>, L<sub>8</sub>, L<sub>22</sub> and Group 6: L<sub>14</sub>, L<sub>15</sub>

# Asian J. Plant Sci., 22 (3): 547-557, 2023

Table 2: Means of ton cane per hectare (TCH) and ton sugar per hectare (TSH) in plant cane and first ration cane at 22 test sites in Thailand

Code			TCH (ton ha <sup>-1</sup> )		TSH (ton ha <sup>-1</sup> )			
	Location	Plant cane	Ratoon cane	Mean	Plant cane	Ratoon cane	Mean	
$\overline{L_1}$	NSN1	116.40	79.68	98.04 <sup>de</sup>	13.07	9.91	11.49°	
$L_2$	KPT1	99.15	72.31	85.73 <sup>fg</sup>	12.77	10.44	11.60°	
$L_3$	SKW1	83.18	71.84	77.51 <sup>h</sup>	9.71	9.87	9.79 <sup>def</sup>	
$L_4$	NMA1	121.78	91.15	106.46e	12.14	11.16	11.65°	
L <sub>5</sub>	NMA2	97.22	84.75	90.99ef	10.10	9.87	9.98 <sup>de</sup>	
L <sub>6</sub>	RYG1	59.03	52.82	55.93 <sup>jk</sup>	6.55	7.18	6.86 <sup>jk</sup>	
L <sub>7</sub>	UDN1	84.32	66.53	75.43 <sup>h</sup>	9.25	8.81	9.03 <sup>efg</sup>	
L <sub>8</sub>	RYG2	53.00	55.63	54.31 <sup>k</sup>	4.88	6.56	5.72i	
L <sub>9</sub>	NSN2	114.21	91.53	102.87sd	13.31	11.07	12.19°	
L <sub>10</sub>	KRI1	91.82	62.49	77.15 <sup>h</sup>	11.20	9.52	10.36 <sup>d</sup>	
L <sub>11</sub>	SPB1	166.43	100.95	133.69ª	19.16	13.66	16.41 <sup>b</sup>	
L <sub>12</sub>	SKW2	53.72	55.12	54.42 <sup>k</sup>	7.63	7.47	7.55 <sup>ij</sup>	
L <sub>13</sub>	KKN1	75.04	84.00	79.52 <sup>gh</sup>	8.90	10.46	9.68 <sup>def</sup>	
L <sub>14</sub>	NMA3	67.82	65.73	66.77 <sup>i</sup>	7.22	8.76	7.99 <sup>hi</sup>	
L <sub>15</sub>	KRI2	59.79	64.92	62.35 <sup>ij</sup>	8.29	9.22	8.76 <sup>fgh</sup>	
L <sub>16</sub>	SPB2	61.48	61.45	61.46 <sup>ijk</sup>	5.77	6.91	6.34 <sup>kl</sup>	
L <sub>17</sub>	PKN1	61.37	74.95	68.16 <sup>i</sup>	6.85	10.43	8.64gh	
L <sub>18</sub>	NSN3	94.43	80.35	87.39 <sup>f</sup>	12.69	11.78	12.23°	
L <sub>19</sub>	NSN4	74.68	98.54	86.61 <sup>fg</sup>	8.60	14.88	11.74°	
L <sub>20</sub>	NSN5	98.35	61.73	80.04 <sup>gh</sup>	11.63	8.10	9.87 <sup>de</sup>	
L <sub>21</sub>	SPB3	129.59	123.06	126.32 <sup>b</sup>	18.11	17.87	17.99ª	
L <sub>22</sub>	PCK1	43.16	49.60	46.38 I	4.91	5.99	5.45 l	
Mean		86.63	74.96	80.80	10.12	10.00	10.06	
F-test				**			**	
CV (%)				14.62			18.41	

CV: Coefficient of variation form Analysis of Variance (ANOVA), F-test: Significantly different by ANOVA, \*\*: Significant at 0.01 probability levels, Means in the same column followed by the same letters were not significantly different by least significant difference (LSD) test at the 0.05 probability level, Tak Fa, Nakhon Sawan (NSN1), Bueng Samakkhi, Khamphaeng Phet (KPT1), Watthana Nakhon, Sa Kaeo (SKW1), Khon Buri, Nakhon Ratchasima (NMA1), Pimai, Nakhon Ratchasima (NMA2), Meuang, Rayong (RYG1), Kumphawapi, Udon Thani (UDN1), Ban Khai, Rayong (RYG2), Kao Liao, Nakhon Sawan (NSN2), Bo Phloi, Kanchanaburi (KRI1), U Thong, Suphan Buri (SPB1), Watthana Nakhon, Sa Kaeo (SKW2), Meuang, Khon Kaen (KKN1), Khong, Nakhon Ratchasima (NMA3), Lao Khwan, Kanchanaburi (KRI2), Dan Chang, Suphan Buri (SPB2), Pranburi, Prachuap Khiri Khan (PKN1), Tak Fa, Nakhon Sawan (NSN3), Kao Liao, Nakhon Sawan (NSN4), Krok Phra, Nakhon Sawan (NSN5), U Thong, Suphan Buri (SPB3) and Pho Thale and Phichit (PCK1)

Table 3: Means and ranks for TCH and TSH of the test genotypes that were obtained from the trials over the 22 original test sites

TCH (ton ha <sup>-1</sup> )		Plant cane		Ratoo	n cane	Average		
Code	Genotypes	Mean	Rank	Mean	Rank	Mean	 Rank	
G1	KK06-501	86.82	3	72.26	6	79.54 <sup>d</sup>	6	
G2	CSB06-5-12	78.42	7	61.81	8	70.11 <sup>e</sup>	7	
G3	CSB06-2-21	83.88	6	75.47	4	79.68 <sup>d</sup>	5	
G4	TBy27-1385	73.89	8	62.90	7	68.40e	8	
G5	TBy28-0348	86.25	4	74.63	5	80.44 <sup>d</sup>	4	
G6	KK3	95.41	2	83.81	2	89.61 <sup>b</sup>	2	
G7	LK92-11	84.97	5	82.65	3	83.81 <sup>c</sup>	3	
G8	Kps01-12	103.43	1	86.14	1	94.78°	1	
LSD 0.05 and df = 990	-	86.63		74.96		80.80**		
TSH (ton ha <sup>-1</sup> )								
G1	KK06-501	9.80	5	9.54	5	9.67 <sup>d</sup>	5	
G2	CSB06-5-12	7.90	8	7.51	8	7.71 <sup>9</sup>	8	
G3	CSB06-2-21	8.96	6	9.45	6	9.21e	6	
G4	TBy27-1385	8.77	7	8.29	7	8.53 <sup>f</sup>	7	
G5	TBy28-0348	10.92	4	10.24	4	10.58 <sup>c</sup>	4	
G6	KK3	11.00	2	11.45	2	11.23 <sup>b</sup>	2	
G7	LK92-11	10.29	3	11.37	3	10.83°	3	
G8	Kps01-12	13.35	1	12.10	1	12.73ª	1	
LSD 0.05 and df = 990		10.12		10.00		10.06**		

LSD: Least significant difference, df: Degree of freedom, \*\*Significant at 0.01 probability levels, Means in the same column followed by the same letters were not significantly different by least significant difference (LSD) test at the 0.05 probability level

Table 4: List of test locations in the individual groups that were derived from cluster analysis based on TCH and TSH of the plant cane and first ratoon cane

	Grouping by hierarchical clustering								
Basis for grouping	Group 6	Group 8	Group 10	Group 12 L <sub>1</sub> , L <sub>4</sub> , L <sub>9</sub>					
Group 1	L <sub>1</sub> , L <sub>4</sub> , L <sub>9</sub>	L <sub>1</sub> , L <sub>4</sub> , L <sub>9</sub>	L <sub>1</sub> , L <sub>4</sub> , L <sub>9</sub>						
Group 2	L <sub>19</sub>	L <sub>19</sub>	L <sub>19</sub>	L <sub>19</sub>					
Group 3	$L_2$ , $L_3$ , $L_5$ , $L_7$ , $L_{10}$ , $L_{13}$ , $L_{18}$ , $L_{20}$	$L_2$ , $L_5$ , $L_{13}$ , $L_{18}$	L <sub>5</sub> , L <sub>13</sub>	L <sub>2</sub> , L <sub>18</sub>					
Group 4	L <sub>11</sub> , L <sub>21</sub>	L <sub>3</sub> , L <sub>7</sub> , L <sub>10</sub> , L <sub>20</sub>	L <sub>11</sub>	$L_5$					
Group 5	L <sub>6</sub> , L <sub>8</sub> , L <sub>12</sub> , L <sub>16</sub> , L <sub>22</sub>	L <sub>11</sub>	L <sub>6</sub> , L <sub>12</sub> , L <sub>16</sub>	L <sub>13</sub>					
Group 6	L <sub>14</sub> , L <sub>15</sub> , L <sub>17</sub>	L <sub>21</sub>	L <sub>14</sub> , L <sub>15</sub> , L <sub>17</sub>	$L_3$ , $L_7$					
Group 7	X	$L_6$ , $L_8$ , $L_{12}$ , $L_{16}$ , $L_{22}$	L <sub>3</sub> , L <sub>7</sub> , L <sub>10</sub> , L <sub>20</sub>	L <sub>10</sub> , L <sub>20</sub>					
Group 8	X	L <sub>14</sub> , L <sub>15</sub> , L <sub>17</sub>	L <sub>21</sub>	L <sub>11</sub>					
Group 9	X	X	L <sub>2</sub> , L <sub>18</sub>	L <sub>21</sub>					
Group 10	X	X	L <sub>8</sub> , L <sub>22</sub>	L <sub>6</sub> , L <sub>12</sub> , L <sub>16</sub>					
Group 11	X	X	X	L <sub>8</sub> , L <sub>22</sub>					
Group 12	X	X	X	L <sub>14</sub> , L <sub>15</sub> , L <sub>17</sub>					
R-square	0.9101	0.9526	0.9725	0.9814					

and  $L_{17}$  (Fig. 1a). Group 1 includes  $L_1$ ,  $L_4$ ,  $L_9$ , Group 2 includes  $L_{19}$ , Group 3 includes  $L_2$ ,  $L_{18}$ ,  $L_5$ ,  $L_{13}$ , Group 4 includes  $L_3$ ,  $L_7$ ,  $L_{10}$ ,  $L_{20}$ , Group 5 includes  $L_{11}$ , Group 6 includes  $L_{21}$ , Group 7 includes  $L_6$ ,  $L_{12}$ ,  $L_{16}$ ,  $L_8$ ,  $L_{22}$  and Group 8 includes  $L_{14}$ ,  $L_{15}$  and  $L_{17}$  (Fig. 1b). The dashed line represents the boundary for the location grouping of 10 groups, with Group 1 including  $L_1$ ,  $L_4$ ,  $L_9$ , Group 2:  $L_{19}$ , Group 3:  $L_5$ ,  $L_{13}$ , Group 4:  $L_{11}$ , Group 5:  $L_6$ ,  $L_{12}$ ,  $L_{16}$ , Group 6:  $L_{14}$ ,  $L_{15}$ ,  $L_{17}$ , Group 7:  $L_3$ ,  $L_7$ ,  $L_{10}$ ,  $L_{20}$ , Group 8:  $L_{21}$ , Group 9:  $L_2$ ,  $L_{18}$  and Group 10 including  $L_{18}$ ,  $L_{22}$  (Fig. 1c). The high R-square obtained suggested that truncation at 12 groups was suitable since location grouping effectively captured the majority of the G-L interaction (Fig. 1d).

Discrimination power and representativeness of test environment (location): The test environments can be divided into the following categories based on the ability of the environment to distinguish between the yield of different genotypes: Group 1 included the L<sub>1</sub>, L<sub>4</sub> and L<sub>9</sub> crops that were grown in the late rainy season (LR) in the central part of Thailand in locations with good soil (loam to clay soil), heavy rainfall and a high OM >1. Group 2 included locations in Thailand's central region, including L<sub>19</sub>, with good soil (clayey soil), high rainfall, high OM >2 and irrigated conditions (EI). Group 3 included L2, L<sub>18</sub> and locations with heavy rainfall, good soil (clayey soil), high OM >1 of rain-fed conditions (ER). Group 4 represents typical environmental conditions found in the northeastern part of Thailand, including L<sub>5</sub>. These parameters include good soil (loamy to sandy soil), high rainfall, high OM >1, cultivation in the late rainy season and rain-fed conditions (LR). In the central region of Thailand, including L<sub>13</sub>, Group 5 represented typical environmental locations with characteristics such as good soil (loamy to sandy soil), high rainfall, high OM >1, of rain-fed conditions (LR). Group 6 represented areas with medium soil (sandy soil), moderate rainfall and high OM >1 of rain-fed conditions (LR) and it consisted of L<sub>3</sub> and L<sub>7</sub>. The locations in Group 7 possessed favorable soil characteristics (clayey soil), high rainfall, high OM >1, were cultivated during the late rainy season and were irrigated (LI), including  $L_{10}$  and  $L_{20}$ . The  $L_{11}$ was a member of Group 8 and stood for areas with good soil (loam to clay soil), little rain, high OM >1 and conditions that were irrigated (LI) when planting took place in the late rainy season. Locations in Group 9 included L<sub>21</sub> and had good soil (loam to clay soil), little rain, a high OM >1 level, early rainy season cultivation and irrigated conditions. Group 10 included  $L_{6}$ ,  $L_{12}$  and  $L_{16}$  locations that have poor soil (clay soil), little rainfall, a low OM 1 and rainfed conditions. Group 11 consisted of rain-fed conditions (LR), comprising L<sub>8</sub> and L<sub>22</sub>, that were planted in the late rainy season in drought-prone areas with poor soil (sandy soil), low rainfall and low soil fertility (OM 0.5). Group 12 consisted of  $L_{14}$ ,  $L_{15}$  and  $L_{17}$ , which were grown in early rainy season rain-fed conditions (ER), represented drought-prone locations, low rainfall and low OM 1.

## Best test environment (location) for sugarcane cultivars:

The test genotypes including Kps01-12 (G8), KK3 (G6), LK92-11 (G7) and TBy28-0348 (G5) that were discovered from the trials at the initial 22 sites had better average cane yields and sugar yields across two crop classes. According to the results of location grouping, the sites within each group that comprised combination crop classes, such as plant cane or ratoon cane, based on TCH or TSH, were divided into 4 sets in a pretty haphazard manner (Table 4). Set 1 (6-Group) chose 6 test sites

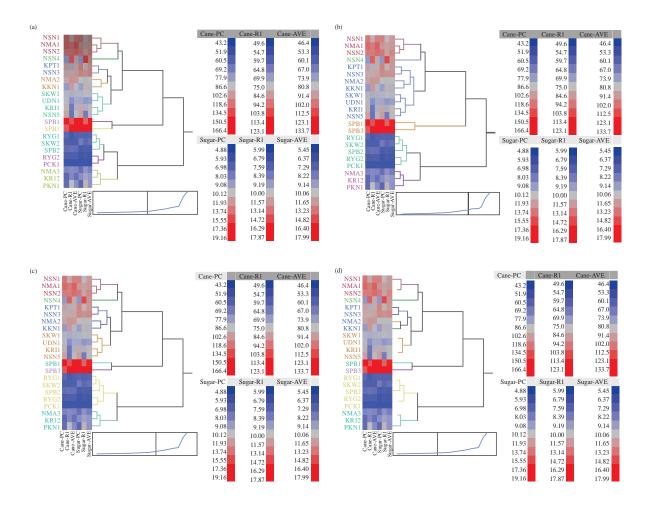


Fig. 1(a-d): Clustering analysis by hierarchical clustering of the 22 test sites for METs of elite sugarcane genotypes in Thailand based on TCH and TSH of plant cane and first ration cane, Dashed line is the cutoff point for location grouping of (a) 6 group, (b) 8 group, (c) 10 group and (d) 12 group

to represent each of the 6 groups, using  $L_4$ ,  $L_{13}$ ,  $L_{14}$ ,  $L_{15}$ ,  $L_{19}$  and  $L_{21}$  as the clustering levels in the hierarchy. Set 2 (8-Group) chose 8 test sites to represent the 8 groups using hierarchical clustering, using the  $L_4$ ,  $L_6$ ,  $L_{13}$ ,  $L_{11}$ ,  $L_{15}$ ,  $L_{19}$ ,  $L_{21}$  and  $L_{22}$  as the groups to be represented. By using hierarchical clustering. Set 3 (10-Group) chose 10 test sites to represent 10 groups:  $L_3$ ,  $L_5$ ,  $L_6$ ,  $L_9$ ,  $L_{11}$ ,  $L_{14}$ ,  $L_{18}$ ,  $L_{19}$ ,  $L_{21}$  and  $L_{22}$ . Set 4 (12-Group) used hierarchical clustering to choose 12 test sites for the representative from 12 groups as  $L_3$ ,  $L_5$ ,  $L_6$ ,  $L_9$ ,  $L_{10}$ ,  $L_{11}$ ,  $L_{13}$ ,  $L_{14}$ ,  $L_{18}$ ,  $L_{19}$ ,  $L_{21}$  and  $L_{22}$  (Table 4).

Nevertheless, some areas more frequently fit into particular categories for both crop classes and yield attributes. Regarding TCH, sets 2 and 3 have a strong correlation (r = 0.976 value), whereas sets 1 and 4 have a high correlation (r = 0.976 value) for TSH (Table 5). This ranking correlation relates the original 22 sites and the four sets grouping test sites, i.e., set 4 picked 12 test sites.

#### Evaluation of the test environment using cane and sugar

yield: The sugarcane experiments' cane and sugar production, as well as their discrimination power, representativeness and attractiveness index, were examined using hierarchical clustering. Based on the test environment's ability to discriminate between different genotypes' yields, the results were standardized and thoroughly analyzed. The following categories apply to the environments tested: Both L<sub>21</sub> (Group 9) grown in the early rainy season and L<sub>11</sub> (Group 8) grown in the late rainy season provide water for all growth phases and had good soil (high fertility) locations that had extremely strong discriminative test conditions. Early drought is affected by growing in Group 11's  $L_8$  and  $L_{22}$ , whereas mid-drought is affected by Group 12's  $L_{14}$ ,  $L_{15}$  and  $L_{17}$ 's early rainy season cultivation. Weak discriminative test environments that depict unfavorable environments were present in both places with limited rainfall and areas with

Table 5: Means over two crop-classes and ranks for TCH and TSH of the test genotypes that were obtained from the trials over the original 22 sites and the 6, 8, 10 and 12 selected test sites in Set 1, Set 2, Set 3 and Set 4

			Set 1 se	elected	Set 2 se	lected	Set 3 se	lected	Set 4 se	elected
	Original 22 sites		6 test site <sup>a</sup>		8 test site <sup>b</sup>		10 test site <sup>c</sup>		12 test sited	
Code										
Cane yield	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
G1	79.54	6	99.47	6	104.10	3	87.41	4	79.81	6
G2	70.11	7	85.08	8	81.04	8	71.92	8	66.82	7
G3	79.68	5	99.74	5	94.98	6	84.51	6	79.93	5
G4	68.40	8	86.38	7	82.81	7	74.41	7	66.59	8
G5	80.44	4	103.47	3	101.63	5	87.05	5	80.76	4
G6	89.61	2	103.14	4	102.78	4	93.00	2	84.43	2
G7	83.81	3	104.32	2	105.62	2	90.28	3	82.63	3
G8	94.78	1	118.17	1	120.32	1	103.30	1	93.24	1
Mean	80.80		99.97		99.16		86.48		79.28	
Rank correlation sugar yield			0.905		0.786		0.905		0.976	
G1	9.67	5	12.62	5	13.08	5	10.91	5	9.68	5
G2	7.71	8	9.62	8	8.88	8	7.97	8	7.14	8
G3	9.21	6	12.32	6	10.75	6	9.49	6	9.15	6
G4	8.53	7	11.59	7	10.38	7	9.46	7	8.16	7
G5	10.58	4	14.29	3	13.69	4	11.70	4	10.70	3
G6	11.23	2	13.68	4	13.70	3	12.33	2	10.36	4
G7	10.83	3	14.58	2	14.38	2	12.12	3	10.80	2
G8	12.73	1	16.33	1	16.76	1	14.13	1	12.74	1
Mean	10.06		13.13		12.70		11.01		9.84	
Rank correlation			0.929		0.976		0.976		0.929	

<sup>a</sup>Set 1 includes L<sub>2</sub>, L<sub>9</sub>, L<sub>11</sub>, L<sub>15</sub>, L<sub>19</sub> and L<sub>21</sub>, <sup>b</sup>Set 2 includes L<sub>4</sub>, L<sub>6</sub>, L<sub>13</sub>, L<sub>11</sub>, L<sub>15</sub>, L<sub>19</sub>, L<sub>21</sub> and L<sub>22</sub>, <sup>c</sup>Set 3 includes L<sub>2</sub>, L<sub>4</sub>, L<sub>7</sub>, L<sub>11</sub>, L<sub>12</sub>, L<sub>13</sub>, L<sub>15</sub>, L<sub>19</sub>, L<sub>21</sub> and L<sub>22</sub>, <sup>d</sup>Set 4 includes L<sub>3</sub>, L<sub>5</sub>, L<sub>6</sub>, L<sub>9</sub>, L<sub>10</sub>, L<sub>11</sub>, L<sub>13</sub>, L<sub>14</sub>, L<sub>18</sub>, L<sub>19</sub>, L<sub>21</sub> and L<sub>22</sub>

rain-fed conditions. With medium to low representativeness, the  $L_6$ ,  $L_{12}$  and  $L_{16}$  (Group 10) test environments may feature unique ecological circumstances that call for more thorough and in-depth experiments in order to choose the best cultivars. The  $L_1$ ,  $L_4$  and  $L_9$  (Group 1) were grown in the late rainy season in the central region,  $L_5$  (Group 4) in the northeast and  $L_2$ ,  $L_{18}$  (Group 3) in the early rainy season, where their high rainfall and good soil (high fertility) locations were the best conditions. Late rainy season cultivation of  $L_3$  and  $L_7$  (Group 6) and late rainy season cultivation of  $L_{10}$  (Group 7) and  $L_{13}$  (Group 5) and irrigated circumstances during  $L_3$  and  $L_7$  (Group 6) and  $L_{13}$  (Group 5) were all relatively perfect conditions. Breeders can choose from  $L_1$ ,  $L_5$ ,  $L_7$ ,  $L_8$ ,  $L_{10}$ ,  $L_{11}$ ,  $L_{13}$ ,  $L_{16}$ ,  $L_{17}$ ,  $L_{18}$ ,  $L_{19}$  and  $L_{21}$  as effective representative test locations in the future to evaluate sugarcane cultivars.

#### **DISCUSSION**

A new sugarcane (*Saccharum* sp. hybrid) cultivar is now developed over the course of 13 years by the Louisiana Sugarcane Variety Development Program (LSVDP) after crossing. The last testing phase consists of outfield trials. An analysis of resource distribution between and within these two testing stages is necessary considering recent developments in pre-outfield testing. In the early

development and mid-growth stages, respectively, of sugarcane, late and early rainy season rain-fed circumstances have a possibility to experience drought. For sugarcane to be able to adapt to drought, it could be important to choose cultivars that do not respond to rainfall the same as KK3 (G6). Breeders may have to select sugarcane types that do not react to soil and rainfall as well as Kps01-12 (G8), which may entail root reaction and growth to absorb water, under lengthy drought situations. Each sugarcane does not respond to the constraints that would allow it to develop deep roots to mentality water in protracted dry situations. The soil types may be a barrier to KK3's ability to develop deep roots because those roots only reach the soil's surface. On the other hand, KK3's ability to swiftly recover from droughts thanks to its ability to root at the soil's surface is a benefit of this trait. With variable numbers of test locations and replications for the two test stages, estimations of the repeatability of sugar output were generated for three different crops. For a certain number of sites and replications, repeatability marginally improved in the pre-outfield tests and slightly reduced in the outfield testing with older crops. The genotype-location yield correlation across test sites showed that there were no redundant test sites. However, there were also significant disparities in the group rankings between certain sites and the decision would change when other subsets of sites were considered. The overall reaction to ration crop years varied very little across groups. Testing across locations is crucial, according to the findings<sup>14</sup>. According to Meena et al.<sup>15</sup> environmental stability revealed the consistency of genotype rankings in each test environment in comparison to the overall rankings of the test settings. Typically, heavy-or light-textured soil tests are used to examine the results of the outfield testing. These genotypes were produced more in locations with clayey soils and less in situations with sandy soils<sup>16</sup>. Reduced stability as the environment changes is caused by the genotype's reaction to a variety of conditions. The performance of sugarcane cultivars in Swaziland's poor-draining (Mhlume) and good-draining (Simunye) soils is influenced by the interplay between soil type and season<sup>17</sup>. Three statistical models were used to examine the effectiveness of this soil categorization system. The findings supported dividing the outfield experiments into groups based on soil texture and showed that there was about equal genotype-by-location interaction within each group of soil as there was between the groups. Pattern analysis was used to examine how sugarcane genotypes in Thailand responded to various settings in terms of sugar yield and its components, cane yield and CCS. To develop effective selection strategies for sugarcane breeding programs, it was intended to give information that might be utilized in this way. The comparison of genotype performance variance across locations and crop years was particularly interesting. Ten sets of sugarcane studies in Thailand are provided with their findings. In general, genotypic responses were more comparable for crop years within sites than for environments across sites. Across several crop years, the relative ranking of genotype groups resulting from cluster analysis was comparable within many locations. However, there were also significant discrepancies in the group rankings between certain sites and the selection would alter across the various subsets of sites considered. The overall reaction to ratoon harvest years varied very little across groups. The results show the value of testing across locations. The homogeneity of the information gathered from several crop years within many locations, however, implies that there may not be much benefit from examining G×E interactions in sugarcane over many crops or years within a single site. Thailand found that five test sites were equally effective in the performance evaluation of sugarcane genotypes as they represented three distinct types of environments: Drought-prone areas (sandy soil, low rainfall), more favorable environments (clay soil, irrigation, early season) and typical late rainy season environments (high rainfall, sandy soil), with the additional

two locations for supplementary sites providing good coverage of the range of environment<sup>18</sup>. While this study found that 22 test sites grouping by hierarchical clustering as 12 groups represent were equally effective in the performance evaluation of sugarcane genotypes as they represent 7 distinct types of environments with drought-prone areas (sandy soil, low rainfall), more favorable environments (clay soil, irrigation, early season) and the additional 5 locations for supplementary. The specific soil characteristics and site circumstances, such as soil types and fertility, indicate the varied environments of Thailand for each group. Due to regions influencing the choice of production seasons and circumstances that rely on the supply of irrigation, production conditions in this study had the least impact on cane output. However, supplementing water at each step may not be sufficient in a drought year. The potential of several locations may be determined as trials that suggested that the environment ought to be extremely diverse, yet considering the outcomes of testing in exceptional kinds, they provide the identical response. The experiment revealed that 12 of the 22 test sites were viable locations for the trial. The results demonstrated that while the rankings of the test genotypes were comparable, the absolute values for both TCH and TSH from the reduced sets were only marginally different from those from the complete set. Nevertheless, future site selection for breeding and assessment will be guided by the insightful deductions established about the nature of the various ecosystems and areas. As a result of selection in various areas' diverse environments, it is possible to develop genetic resistance to scarce water supply, according to the variety and severity of water stress within regions<sup>19</sup>. The number of regional trails might be decreased by removing those that relate to one another through indirect selection among settings, which would help save and utilize resources more effectively<sup>20</sup>. The findings demonstrated that although the rankings of the test genotypes were comparable, the absolute values for both cane yield and sugar yield from the reduced sets were only marginally different from those from the complete set. In all four scenarios, it is advised to have 4: 3: 3: 2 (LR: ER: LI: EI) test sites. The researchers concluded that a breeding program would be more effective if there were fewer outfield test locations and more outfield test subjects overall.

Estimates of the possible cane yields over years and locales suggested that testing across sites may take years, thereby speeding up the process of identifying elite clones. To cover most of the nation's production areas, test sites should differ in terms of climate (soil type, soil fertility, rainfall and irrigation), geography (landscape) and circumstances (rainfall and irrigation). For subsequent usage

in sugarcane evaluation, the test sites should be chosen as samples of various production areas that are organized into similar clusters. The soil texture element has a significant impact on yield expression and in this test, 22 locations represent test sites on yield trials for sugarcane production in Thailand, which include both less appropriate and more suitable areas. The selection of representative test sites significantly minimizes the number of pointless test sites used in the assessment of sugarcane, which, in turn, lowers the cost and shortens the duration of the METs for sugarcane in Thailand. The effectiveness of sugarcane breeding programs depends on the evaluation of sugar cane at a smaller number of test sites by removing unneeded test sites.

#### CONCLUSION

Based on the findings, it has been stated that Kps 01-12 and KK3 genotypes had the highest mean yield and outstanding stability in the investigated environment. It was suggested that the location grouping be truncated at 12 groups to adequately capture most of the  $G \times L$  interaction. The METs of sugarcane genotypes in Thailand could potentially be improved by minimizing the 22 locations to 12 sites, which would significantly save time and resources.

#### SIGNIFICANCE STATEMENT

releasing a new cultivar, sugarcane genotypes must undergo expensive multi-environment tests (METs), which require adequate test sites to increase cost-effectiveness. To create efficient representative ecological zoning division, the METs of sugarcane genotypes might be enhanced. The high R-square identified recommended truncation at 12 groups since location grouping can effectively capture the majority of  $G \times L$ interactions. By minimizing the 22 locations to 12, it would be reasonable to further improve the METs of sugarcane genotypes in Thailand. This study's effective and representative ecological zone division makes it possible to choose a useful cultivar for sugarcane. Improvements to the sugarcane genotypes' METs might result in considerable time and cost savings.

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