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## Research Article

# Correlation Between Rapid Measurement and Leaf Chlorophyll Content of Various Sugarcane Genotypes at Different Growth Phases

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## Abstract

**Background and Objective:** Chlorophyll is an essential pigment for plant photosynthesis and a key factor in crop development and yield. Therefore, this research aimed to compare the SPAD chlorophyll meter reading (SCMR) and chlorophyll content of sugarcane varieties under natural field conditions and to determine the relationship between chlorophyll content and SCMR at different growth stages. **Materials and Methods:** A Randomized Complete Block Design (RCBD) with 4 replications was used and 27 sugarcane genotypes were assigned as treatments. The chlorophyll content, including the total chlorophyll (Chl total), Chlorophyll a (Chl a) and Chlorophyll b (Chl b) contents and SCMR were measured during the tillering, elongation and physiological maturity phases. **Results:** The KK07-599, 95-127 and BM500-025 revealed outstandingly high SCMR, Chl total and Chl a during the tillering, elongation and physiological maturity phases. The correlation between SCMR and Chl a was positive during the tillering, elongation and physiological maturity phases and SCMR existed in the Chl total. In addition, there was a positive correlation between Chl a and Chl total in all three phases. **Conclusion:** The rapid measurement of the SCMR can be used as an indirect measurement of Chl a and Chl total during the tillering, elongation and physiological maturity phases of sugarcane. This would be advantageous for evaluating sugarcane genotypes with limits in a large population and for avoiding destructive sampling.

**Key words:** SCMR, Chl a, Chl b, Chl total, rapid measurement, growth stage

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**Competing Interest:** The authors have declared that no competing interest exists.

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

## INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is an important economic crop in many countries. At present, sugarcane is grown in a production system that has encountered climate change, which poses a major problem, especially abiotic and biotic stress in tropical areas. In this context, a sugarcane variety with a high capacity for photosynthesis is preferable<sup>1,2</sup>. Photosynthesis and chlorophyll content are significantly positively correlated<sup>3</sup>.

Chlorophyll is an important pigment in photosynthesis consisting of chlorophyll a and chlorophyll b, whose functions are to absorb light and transmit solar energy<sup>4</sup>. The amount of pigment in leaves is used to indicate sugarcane growth<sup>5</sup>. The elongation phase is the most important stage of sugarcane growth<sup>6-8</sup> and yield productivity is determined during this phase<sup>9</sup>. This period occurs between 120 and 270 days after planting, during which the sugarcane can grow up to 4-5 inches per month<sup>10</sup>. Consequently, good growth development during the elongation period allows sugarcane to yield effectively<sup>11</sup>. However, both indirect and direct methods are available for determining the chlorophyll content of sugarcane leaves<sup>12</sup>. The chlorophyll content is directly measured by destructive sampling and using a spectrophotometer to extract leaf tissue<sup>13</sup>. Although, this approach is very precise, the direct method approach is expensive, labor intensive and time-consuming and requires destructive sampling, making it inappropriate for large-scale plant observation in the field<sup>8</sup>. Additionally, a SPAD chlorophyll meter reading (SCMR) instrument can be utilized to measure chlorophyll content indirectly; this method enables rapid data collection, saves time and does not require destructive sampling<sup>14</sup>. Nevertheless, the precision of this method is limited and it cannot differentiate between quantities of different kinds of chlorophyll. The chlorophyll content and SCMR have been evaluated in sugarcane and a positive correlation has been reported from 8 to 12 months after planting (MAP)<sup>3</sup>. Sugar accumulation and the physiological maturity phase of sugarcane are determined as 8-12 MAP<sup>9</sup>. During this period, the sugarcane growth rate declines and sucrose begins to accumulate in the stalks<sup>15</sup>. Sugar obtained from photosynthesis is stored in the stalks<sup>16</sup>. Hence, sugarcane is not particularly concerned with the growth of sugarcane stems during the physiological maturity period. A previous report by Jangpromma *et al.*<sup>17</sup> found a relationship between SCMR and sugarcane chlorophyll content under a 10-day drought in the early growth stage at 3 MAP or during the tillering phase in pot conditions.

The chlorophyll measurements are typically used under drought conditions to identify drought resistance in sugarcane<sup>18</sup>.

However, there is a lack of literature on tillering, elongation and physiological maturity phases under natural field conditions and further research is needed to understand the relationship between direct and indirect approaches in these conditions. Therefore, this research aimed to compare the SCMR and chlorophyll content traits of 27 sugarcane varieties under natural field conditions and to determine the relationship between chlorophyll content and the SCMR of different sugarcane varieties during the tillering, elongation and physiological phases. This information will be useful in understanding the potential of techniques involving chlorophyll content in connection with sugarcane photosynthesis at various growth phases.

## MATERIALS AND METHODS

**Plant materials and experimental details:** The 27 sugarcane varieties, namely 85-2-352, 95-127, BM500-025, F03-362, F152, K84-200, K88-92, KK07-250, KK07-599, KK08-0358, KK087-037, KK09-0939, KK12R-076, KK13-288, LK92-11, LKK08-059, Malaysia, Si Samrong 1, Singapore, SP50, SP72, SP80, TPJ04-768, UT3, UT4, UT5 and UT6, were used. A field experiment was conducted under rain-fed conditions at a field crop research station, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand, from December 2020 to October 2021. A Randomized Complete Block Design (RCBD) with four replications was used in this experiment. Plastic bags were used to prepare the sugarcane seedlings. Thirty days after planting, only uniform and normal plantlets were chosen and transplanted to the field plot. The soil type was the Yasothon series (fine-loamy; siliceous, isohypothermic, Oxic Paleustults). The plots were 6×6 m<sup>2</sup>, with 50 cm spacing between plants and 150 cm spacing between rows. Two applications of a chemical fertilizer formula comprised of 50 kg N/ha, 50 kg P<sub>2</sub>O<sub>5</sub> and 50 kg K<sub>2</sub>O ha were made as follows: (1) Base dressing on the date of transplant and (2) Top dressing at 4 MAP or the tillering stage. The free-hand weeding method was used to control the weeds during the initial vegetative stage. Throughout the experiment, there was no significant insect or disease breakout; thus, no chemical pesticide was used.

**Data collection:** The chlorophyll content was measured in three plants from each plot taken from the second fully expanded leaf of the main stem in the middle position.

Leaf discs (1 cm<sup>2</sup> each) were cut from the leaf blade samples. Measurements of leaf chlorophyll were made during the tillering phase (3 MAP), elongation phase (6 MAP) and physiological maturity phase (9 MAP) between 09:00 and 11:00 am. A spectrophotometer (Jenway 7315 spectrophotometer by Bibby Scientific Ltd., Stone, Staffe, UK) was used to measure the absorbance (A) of 3 mL extracts at 647 and 664 nm after some of the leaf discs were incubated in 5 mL N, N-Dimethylformamide (DMF) for 48 hrs in the dark. The following calculations were made by Jangpromma *et al.*<sup>17</sup> for Chl total, Chl a and Chl b contents:

$$\text{Chl a} = 12.64 A_{664} - 2.99 A_{647}$$

$$\text{Chl b} = -5.6 A_{664} + 23.24 A_{647}$$

$$\text{Chl total} = 7.04 A_{664} + 20.27 A_{647}$$

The SCMR was observed 3 times during the tillering phase (3 MAP), elongation phase (6 MAP) and physiological maturity phase (9 MAP) on the second expanded leaf from the top of the main stem. The SCMR was measured using a SPAD-502 m (Minolta SPAD-502 m, Tokyo, Japan). The data were averaged at three positions along the length of the leaf blade in each replication.

**Statistical analysis:** The collected data were subjected to One-way Analysis of Variance (ANOVA) according to an RCBD using Statistix 10. The comparison among genotypes for all parameters was performed based on the least significant difference (LSD) test<sup>19</sup>. The simple correlation established the relationships between SCMR and leaf chlorophyll content, i.e., Chl a, Chl b and Chl total.

## RESULTS

**SPAD chlorophyll meter reading (SCMR) and leaf chlorophyll content parameters:** The 27 sugarcane varieties had different SCMR responses during the tillering, elongation and physiological maturity phases. During the tillering phase, six sugarcane varieties, i.e., KK07-599, Singapore, 95-127, F03-362, BM500-025 and UT6, had high SCMR values. The K84-200 revealed a rather low SCMR value compared with the other varieties during the tillering phase (Fig. 1a). The KK07-599, 95-127, F03-362, BM500-025, UT6 and 85-2-352 showed outstanding SCMR responses during the elongation phase, whereas F152 had a low SCMR during the elongation phase (Fig. 1b). The SCMR values of KK07-599, 95-127, BM500-025, SP50 and 85-2-352 were high during the physiological maturity phase. The K84-200 and LK92-11 had low SCMR

values during the physiological maturity phase (Fig. 1c). Moreover, three sugarcane varieties, namely KK07-599, 95-127 and BM500-025, showed an outstanding SCMR during the three developmental periods (Fig. 1).

The leaf chlorophyll content, i.e., Chl a, Chl b and Chl total, of the 27 sugarcane varieties differed during the tillering, elongation and physiological maturity phases (Table 1). During the tillering period, sugarcane varieties 95-127, LK92-11 and KK07-599 had a high Chl a. The KK07-250, SP80 and KK07-599 had a high Chl b and in KK07-599, K88-92, LK92-11, 95-127, BM500-025 and TPJ04-768 had a high Chl total during the tillering phase (Table 1). During the elongation phase, K88-92, KK07-599 and 95-127 had a high Chl a, while LKK08-059, KK08-0358 and KK07-250 had a high Chl b during the elongation phase. The K88-92, 85-2-352, SP50, BM500-025 and 95-127 had a high Chl total at this phase (Table 1). During the physiological maturity phase, KK07-599, 85-2-352, BM500-025 and SP50 had a high Chl a, whereas SP50, TPJ04-768 and SP80 had a high Chl b. The KK07-599, SP50 and 85-2-352 showed a high Chl total during the physiological maturity phase (Table 1). Therefore, sugarcane varieties KK07-599, 95-127 and BM500-025 represented outstanding Chl a and Chl total values during the tillering and elongation phases. Furthermore, KK07-599 revealed a significantly higher Chl a and Chl total during the physiological maturity phase. However, the response of Chl b varied among sugarcane varieties during the tillering, elongation and physiological maturity phases. The KK07-250, LKK08-059 and SP50 showed an outstandingly high Chl b during the tillering, elongation and physiological maturity phases, respectively (Table 1).

### Correlation between SCMR and leaf chlorophyll content:

The 27 sugarcane varieties showed a positive relationship between SCMR and Chl a during the tillering, elongation and physiological maturity phases (Fig. 2a-c). In contrast, SCMR was not correlated with Chl b during the three measurement phases (Fig. 2d-f). Furthermore, the relationship between SCMR and Chl total was significantly related during the tillering, elongation and physiological phases (0.643, 0.696 and 0.794, respectively) (Fig. 2g-i).

The correlation between Chl a and Chl b was not related during the tillering, elongation, or physiological maturity phases (Fig. 3a-c). Nevertheless, the relationship between Chl a and Chl total showed a high correlation during the tillering, elongation and physiological maturity phases (0.925, 0.870 and 0.947, respectively) (Fig. 3d-f). In addition, the Chl b values of the 27 sugarcane varieties were not related to Chl total during the tillering phase (Fig. 3g), but during the

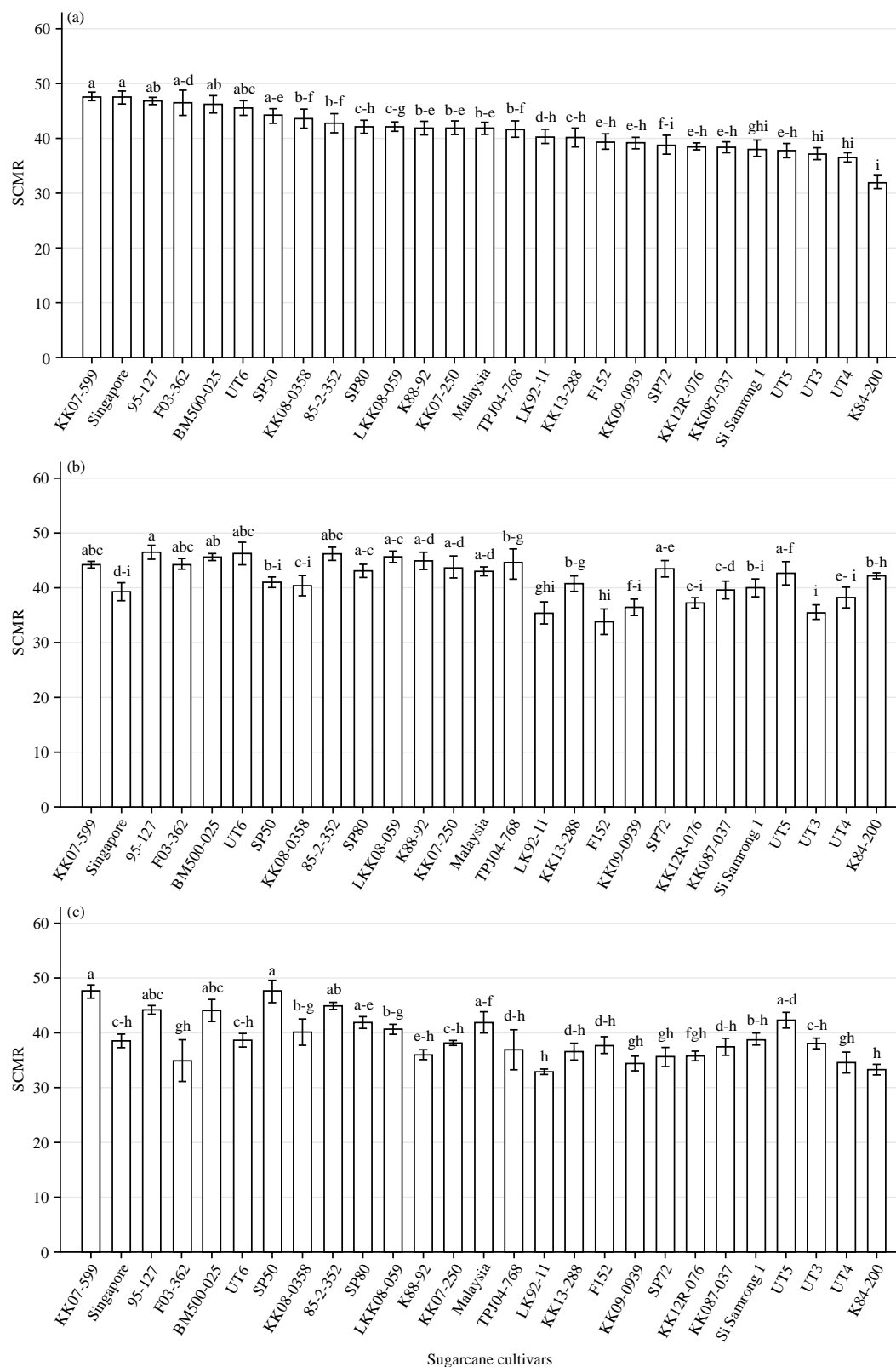


Fig. 1(a-c): SPAD chlorophyll meter reading (SCMR) of 27 sugarcane varieties during the, (a) Tillering, (b) Elongation and (c) Physiological maturity phases

Table 1: Leaf chlorophyll content ( $\mu\text{g}/\text{cm}$ ), i.e., chlorophyll a (Chl a), chlorophyll b (Chl b) and the total chlorophyll (Chl total) of 27 sugarcane varieties during the tillering, elongation and physiological maturity phases

Sugarcane varieties	Tillering phase			Elongation phase			Physiological maturity phase		
	Chl a	Chl b	Chl total	Chl a	Chl b	Chl total	Chl a	Chl b	Chl total
KK07-599	9.3 <sup>abc</sup>	1.3 <sup>abc</sup>	10.6 <sup>a</sup>	10.5 <sup>ab</sup>	2.3 <sup>hi</sup>	12.9 <sup>abcdef</sup>	15.2 <sup>a</sup>	1.1 <sup>bcd</sup>	14.6 <sup>a</sup>
Singapore	5.8 <sup>ghij</sup>	1.3 <sup>abcd</sup>	7.1 <sup>cdefg</sup>	8.4 <sup>cdefgh</sup>	4.0 <sup>abcdef</sup>	13.0 <sup>abcde</sup>	8.2 <sup>cd</sup>	1.3 <sup>abcd</sup>	8.4 <sup>gh</sup>
95-127	10.5 <sup>a</sup>	-0.3 <sup>jk</sup>	10.2 <sup>ab</sup>	10.1 <sup>abc</sup>	3.7 <sup>bcdefg</sup>	13.8 <sup>abc</sup>	11.6 <sup>bc</sup>	0.8 <sup>e</sup>	11.6 <sup>bcde</sup>
F03-362	7.1 <sup>cdefghi</sup>	1.2 <sup>abcdef</sup>	8.3 <sup>abcdef</sup>	8.4 <sup>cdefghi</sup>	4.4 <sup>abcd</sup>	12.5 <sup>bcdefg</sup>	7.8 <sup>d</sup>	1.1 <sup>bcd</sup>	8.9 <sup>efgh</sup>
BM500-025	9.2 <sup>abcde</sup>	0.2 <sup>hij</sup>	9.4 <sup>abc</sup>	9.8 <sup>abcd</sup>	4.0 <sup>abcde</sup>	13.8 <sup>abc</sup>	11.7 <sup>abc</sup>	1.0 <sup>bcd</sup>	11.0 <sup>bcdefg</sup>
UT6	8.4 <sup>abcdef</sup>	0.6 <sup>defghi</sup>	9.0 <sup>abcd</sup>	8.0 <sup>defghijk</sup>	3.6 <sup>cdefg</sup>	11.8 <sup>cdefg</sup>	9.0 <sup>bcd</sup>	1.1 <sup>bcd</sup>	10.1 <sup>defgh</sup>
SP50	5.4 <sup>hij</sup>	0.7 <sup>cdefghi</sup>	6.0 <sup>fg</sup>	9.8 <sup>abcd</sup>	4.5 <sup>abcd</sup>	14.3 <sup>ab</sup>	11.7 <sup>abc</sup>	1.9 <sup>a</sup>	13.7 <sup>ab</sup>
KK08-0358	7.7 <sup>bcdefgh</sup>	1.1 <sup>bcdef</sup>	8.8 <sup>abcde</sup>	6.4 <sup>ijkl</sup>	4.8 <sup>ab</sup>	11.1 <sup>efghi</sup>	10.0 <sup>bcd</sup>	0.9 <sup>cde</sup>	10.9 <sup>bcdefg</sup>
85-2-352	8.0 <sup>bcdefg</sup>	0.3 <sup>hij</sup>	8.3 <sup>abcdef</sup>	10.0 <sup>abcd</sup>	4.3 <sup>abcd</sup>	14.4 <sup>ab</sup>	12.1 <sup>ab</sup>	1.1 <sup>bcd</sup>	13.3 <sup>abc</sup>
SP80	7.2 <sup>cd</sup>	1.4 <sup>ab</sup>	8.5 <sup>abcde</sup>	9.3 <sup>abcdef</sup>	3.7 <sup>bcdefg</sup>	12.0 <sup>cdefg</sup>	9.3 <sup>bcd</sup>	1.4 <sup>abc</sup>	10.7 <sup>cdefg</sup>
LKK08-059	7.7 <sup>bcdefgh</sup>	1.1 <sup>bcdefg</sup>	8.8 <sup>abcde</sup>	8.4 <sup>cdefghi</sup>	5.0 <sup>a</sup>	13.7 <sup>abcd</sup>	9.4 <sup>bcd</sup>	1.3 <sup>bcd</sup>	10.7 <sup>cdefg</sup>
K88-92	9.2 <sup>abcd</sup>	1.1 <sup>bcdefg</sup>	10.3 <sup>ab</sup>	11.1 <sup>a</sup>	4.2 <sup>abcde</sup>	14.7 <sup>a</sup>	8.9 <sup>bcd</sup>	1.1 <sup>bcd</sup>	10.0 <sup>defgh</sup>
KK07-250	6.0 <sup>efghij</sup>	1.8 <sup>a</sup>	7.7 <sup>cdefg</sup>	8.2 <sup>cdefghij</sup>	4.7 <sup>abc</sup>	12.4 <sup>bcdefg</sup>	9.5 <sup>bcd</sup>	1.1 <sup>bcd</sup>	10.6 <sup>cdefgh</sup>
Malaysia	7.9 <sup>bcdefg</sup>	-0.6 <sup>k</sup>	7.4 <sup>cdefg</sup>	8.3 <sup>cd</sup>	3.5 <sup>defgh</sup>	12.3 <sup>bcdefg</sup>	7.6 <sup>d</sup>	1.2 <sup>bcd</sup>	8.8 <sup>fgh</sup>
TPJ04-768	7.7 <sup>bcdefgh</sup>	1.3 <sup>abcde</sup>	9.0 <sup>abc</sup>	7.6 <sup>efghijkl</sup>	3.5 <sup>defgh</sup>	10.8 <sup>ghij</sup>	7.4 <sup>d</sup>	1.5 <sup>ab</sup>	8.9 <sup>efgh</sup>
LK92-11	9.7 <sup>ab</sup>	0.6 <sup>efghi</sup>	10.3 <sup>ab</sup>	7.2 <sup>ghijkl</sup>	4.2 <sup>abcde</sup>	11.0 <sup>efghi</sup>	7.9 <sup>d</sup>	1.1 <sup>bcd</sup>	8.9 <sup>efgh</sup>
KK13-288	8.0 <sup>bcdefg</sup>	-0.3 <sup>jk</sup>	7.7 <sup>cdefg</sup>	9.1 <sup>bcdefg</sup>	3.8 <sup>abcdefg</sup>	12.9 <sup>abcdef</sup>	9.7 <sup>bcd</sup>	1.2 <sup>bcd</sup>	11.5 <sup>bcdef</sup>
F152	8.3 <sup>abcdef</sup>	0.3 <sup>hij</sup>	8.7 <sup>abcde</sup>	9.5 <sup>abcde</sup>	3.6 <sup>cdefg</sup>	12.4 <sup>bcdefg</sup>	10.0 <sup>bcd</sup>	1.3 <sup>bcd</sup>	11.8 <sup>abcd</sup>
KK09-0939	6.8 <sup>efghij</sup>	1.1 <sup>bcdefg</sup>	7.9 <sup>bcdef</sup>	6.5 <sup>hijkl</sup>	3.1 <sup>efgh</sup>	9.6 <sup>hijk</sup>	8.2 <sup>cd</sup>	0.9 <sup>cde</sup>	9.0 <sup>defgh</sup>
SP72	5.15 <sup>j</sup>	0.3 <sup>hij</sup>	5.4 <sup>g</sup>	8.3 <sup>cdefghij</sup>	3.4 <sup>defgh</sup>	11.7 <sup>defg</sup>	7.6 <sup>d</sup>	1.1 <sup>bcd</sup>	8.8 <sup>fgh</sup>
KK12R-076	6.2 <sup>efghij</sup>	0.4 <sup>ghi</sup>	6.6 <sup>defg</sup>	5.8 <sup>l</sup>	2.4 <sup>hi</sup>	8.1 <sup>k</sup>	8.2 <sup>cd</sup>	1.1 <sup>bcd</sup>	9.3 <sup>defgh</sup>
KK087-037	6.8 <sup>efghij</sup>	0.3 <sup>hij</sup>	7.1 <sup>cdefg</sup>	9.2 <sup>abcdefg</sup>	1.7 <sup>i</sup>	10.5 <sup>ghij</sup>	7.7 <sup>d</sup>	0.9 <sup>cde</sup>	8.6 <sup>gh</sup>
Si Samrong 1	6.0 <sup>efghij</sup>	0.1 <sup>jk</sup>	6.0 <sup>fg</sup>	7.5 <sup>efghijkl</sup>	3.3 <sup>defgh</sup>	11.2 <sup>efgh</sup>	7.4 <sup>d</sup>	0.9 <sup>cde</sup>	8.3 <sup>gh</sup>
UT5	6.8 <sup>defghij</sup>	0.6 <sup>efghi</sup>	7.4 <sup>cdefg</sup>	9.3 <sup>abcdef</sup>	3.7 <sup>bcdefg</sup>	13.0 <sup>abcde</sup>	10.4 <sup>bcd</sup>	1.1 <sup>bcd</sup>	11.6 <sup>bcdef</sup>
UT3	6.4 <sup>efghij</sup>	0.8 <sup>bcdefgh</sup>	7.1 <sup>cdefg</sup>	6.4 <sup>ijkl</sup>	2.8 <sup>efghi</sup>	9.1 <sup>ijk</sup>	8.9 <sup>bcd</sup>	1.1 <sup>bcd</sup>	10.0 <sup>defgh</sup>
UT4	5.8 <sup>ghij</sup>	0.6 <sup>efghi</sup>	6.4 <sup>efg</sup>	6.1 <sup>kl</sup>	2.7 <sup>ghi</sup>	8.8 <sup>jk</sup>	7.9 <sup>d</sup>	1.0 <sup>bcd</sup>	8.8 <sup>fgh</sup>
K84-200	4.6 <sup>j</sup>	0.8 <sup>bcdefgh</sup>	5.4 <sup>g</sup>	9.2 <sup>abcdefg</sup>	3.8 <sup>abcdefg</sup>	13.0 <sup>abcde</sup>	6.8 <sup>d</sup>	1.0 <sup>bcd</sup>	7.8 <sup>h</sup>
Mean	7.3	0.7	8	8.4	3.6	12	9.2	1.1	10.2
F-test	**	**	**	**	**	**	**	**	**

elongation phase, Chl b was correlated to Chl total (0.620) (Fig. 3h). During the physiological maturity phase, Chl b was not correlated to Chl total (Fig. 3i).

## DISCUSSION

Sugarcane production faces climate change, which is a major problem worldwide<sup>20</sup>. In addition, sugarcane is grown under rain-fed conditions in tropical areas, including Thailand<sup>21,22</sup>, which influences drought at the initial stage of sugarcane development<sup>23</sup>. Furthermore, several environmental conditions, including temperature, light intensity, atmospheric CO<sub>2</sub> concentration and water availability, affect photosynthesis<sup>24,25</sup>. A sugarcane variety with a high yield indicates great potential for photosynthesis<sup>26,27</sup>. Chlorophyll is a pigment in leaves that has been associated with photosynthesis in plants and is essential for crop productivity<sup>3</sup>. Moreover, sugarcane's capacity to resist drought is closely related to how well adapted it is to drought in terms of SCMR and chlorophyll content<sup>3,17,28</sup>.

The SCMR is an assessment of the photosynthetically active light-transmittance traits in the leaf<sup>29,30</sup>, which is a property related to the concept of photosynthetic rate; however, it is an indirect measurement<sup>18</sup>. Thus, a destructive sample is obtained and leaf tissue is extracted and measured using a spectrophotometer to directly determine the chlorophyll concentration<sup>13</sup>. An indirect measurement of the chlorophyll content in the leaf blade is the SCMR<sup>31</sup> and it provides a rapid and accurate approach for identifying and measuring sugarcane resistant to drought conditions<sup>32,33</sup>. In this research, 27 sugarcane varieties had different SCMR responses. Three sugarcane varieties, i.e., KK07-599, 95-127 and BM500-025, revealed an outstanding SCMR during the tillering, elongation and physiological maturity phases (Fig. 1). The responses to SCMR among the sugarcane varieties used in this study are consistent with Bhavana *et al.*<sup>33</sup>, who noted that the SCMR values varied significantly among sugarcane varieties. Radhamani *et al.*<sup>31</sup> showed that sugarcane varieties had different SCMR responses at different growth stages; furthermore, SCMR was reduced during the development

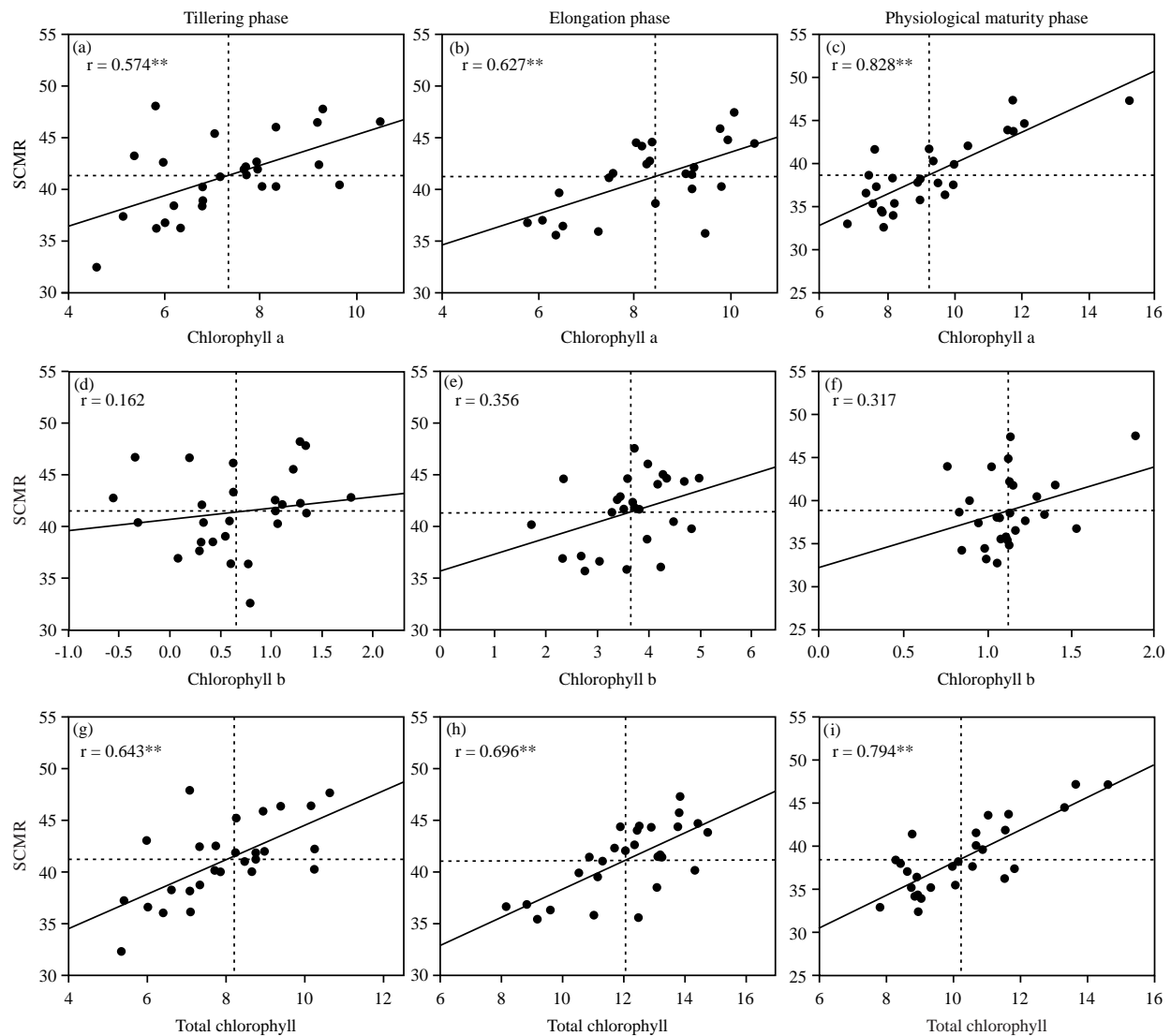


Fig. 2(a-i): Correlation between SPAD chlorophyll meter reading (SCMR) and leaf chlorophyll content, i.e., chlorophyll a (Chl a), chlorophyll b (Chl b) and the total chlorophyll (Chl total), of 27 sugarcane varieties during the tillering, elongation and physiological maturity phases (n = 25)

stage. Ten sugarcane varieties showed different SCMR values under well-watered conditions at 90 days after transplant (DAT) or the tillering phase, while they had a reduced SCMR after 10 days of drought or 110 DAT<sup>17</sup>. Moreover, sugarcane varieties had a high SCMR, indicating that they were classified as drought-resistant varieties<sup>33,34</sup>.

During the tillering phase, sugarcane clones under normal conditions revealed an increasing trend in the SCMR up to 120 days after planting (DAP) and some clones showed a decrease in the SCMR of less than 50 SPAD units<sup>34</sup>. Compared to the other stages of sugarcane growth, SCMR had a stronger

correlation with productivity during the tillering stage<sup>31</sup>. The SCMR value obtained by Chumphu *et al.*<sup>30</sup> showed no difference in SCMR among sugarcane elite clones in ratoon crops at 90 days after harvest (DAH) or the tillering phase, whereas there was a significant difference in SCMR at 180 (elongation phase) and 270 DAH (physiological maturity phase).

The chlorophyll content, including Chl a, Chl b and Chl total, showed different responses during the three phases (Table 1). Oliveros *et al.*<sup>12</sup> explained that the variance in chlorophyll concentration, including Chl a, Chl b and Chl total,



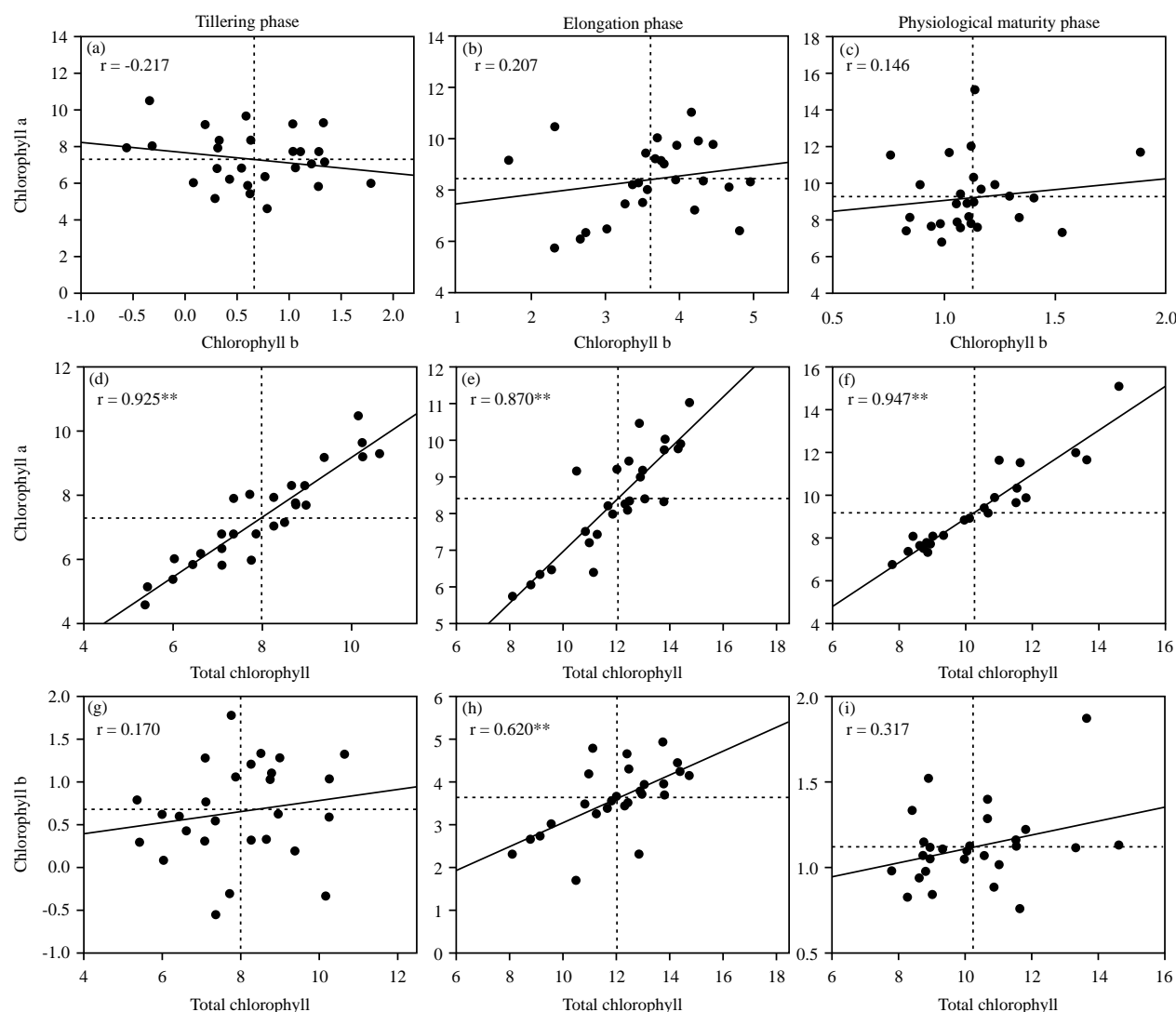


Fig. 3(a-i): Correlation between chlorophyll a (Chl a), chlorophyll b (Chl b) and the total chlorophyll (Chl total) of 27 sugarcane varieties during the tillering, elongation and physiological maturity phases (n = 25)

was influenced by physiological development. In this result, KK07-599 showed the highest Chl a and Chl total during the physiological maturity phase, followed by the elongation and tillering phases (Table 1). In contrast, the greatest total chlorophyll concentration was found during the tillering stage, followed by the elongation and maturity phases<sup>31</sup>. Genetics might have an impact on this difference. In addition, short- and long-term drought periods have reduced leaf chlorophyll contents in plant canes and they are usually greater in plant canes than in ratoon canes<sup>23</sup>.

The different sugarcane varieties had various responses to Chl b during the tillering, elongation and physiological

maturity phases (Table 1). There was a significant difference in Chl b before the elongation stage and before harvest<sup>35</sup>. However, the behavior of Chl a and b exhibited the same chromatographic pattern but with differing concentrations<sup>36</sup>. Chlorophyll quantification is thought to be an essential indicator for confirming the concentration of pigments involved in the transfer of energy and the absorption of sunlight during the photochemical process of photosynthesis<sup>25</sup>. In addition to absorbing photosynthetically active light, it proceeds to the reaction centers of PS I and II, where the quantum photosynthetic process is converted via chlorophyll and accessory pigments of chlorophyll-protein



complexes<sup>17,32,33</sup>. The process of photosynthesis is the basis for yield development<sup>11</sup>. Moreover, drought-resistant sugarcane varieties had a greater chlorophyll concentration than drought-susceptible varieties<sup>17</sup>.

The SCMR was positively correlated with Chl a and Chl total, but it was not related to Chl b during the tillering, elongation and physiological maturity phases (Fig. 2). These results are consistent with Jangpromma *et al.*<sup>17</sup>, who showed a positive correlation between SCMR and chlorophyll content under drought during the early growth development stage and the tillering phase. In addition, the SCMR of sugarcane seedlings was highly correlated to Chl a and Chl total and was related to Chl b<sup>12</sup>. During the physiological maturity phase, the current research agrees with Bunphan *et al.*<sup>3</sup>, who reported that 15 sugarcane varieties showed a positive correlation between SCMR and chlorophyll content under rain-fed conditions at 8-9 MAP and that SCMR showed a strong correlation with sugar yield and cane yield. In addition, SCMR and chlorophyll content were also associated with sugarcane productivity<sup>31</sup>.

In the current study, there were positive correlations between Chl a and Chl total during the tillering, elongation and physiological maturity phases (Fig. 3). This agrees with de Almeida Silva *et al.*<sup>2</sup>, who studied two sugarcane genotypes under drought conditions and indicated that Chl a was related to Chl total. The Chl a and Chl total were not associated with Chl b during the three phases (Fig. 3). The Chl a and Chl b had a moderate correlation before the dry season and before harvest<sup>35</sup>. Furthermore, two sugarcane varieties showed a positive relationship between Chl b and Chl total under water-deficit conditions<sup>2</sup>. In two sugarcane varieties, Chl b content was greater than Chl a under salt stress conditions and the concentration of Chl total dropped<sup>36</sup>. Under normal conditions, however, the Chl a content was greater than the concentration of Chl b<sup>37</sup>. About 75% of chlorophyll in green plants is composed of Chl a<sup>35</sup>. Manarim and de Aguiar<sup>36</sup> and Tomo *et al.*<sup>38</sup> indicated that most Chl a exists in nature and is essential to photochemical reactions in the photosynthesis process because it contributes electrons. The Chl a is a substance present in complex photosynthetic cells and cyanobacteria as blue-green algae, while a supplementary pigment found in plants and other complex photosynthetic organisms is known as Chl b<sup>35</sup>. Furthermore, light energy of a different wavelength is absorbed by Chl b and transferred to Chl a for eventual conversion to chemical energy<sup>35</sup>. Pigment in leaves has a connection to photosynthesis and is crucial for crop efficiency<sup>3</sup>. Nevertheless, a combination of various factors, including an efficient C4 photosynthetic system, provides sugarcane with the ability to produce high biomass and

yield<sup>25</sup>. Furthermore, these approaches might be complicated depending on the sugarcane genotype and its developmental stage<sup>2</sup>.

## CONCLUSION

The comparison of 27 sugarcane varieties showed different SCMR, Chl a, Chl b and Chl total during the tillering, elongation and physiological phases under natural field conditions. The KK07-599, 95-127 and BM500-025 revealed outstandingly high SCMR, Chl a and Chl total during the three phases. In terms of the relationship between non-destructive and destructive measurements, SCMR had a positive correlation with Chl a and Chl total at all three phases. In addition, the association between Chl a and Chl total existed in all three phases. Therefore, a rapid measurement, such as SCMR, can be used as an indirect measurement of Chl a and Chl total during tillering, elongation and physiological maturity phases.

## SIGNIFICANCE STATEMENT

This study discovers a rapid measurement, such as SCMR, can be used as an indirect measurement of Chl a and Chl total during tillering, elongation and physiological maturity phases that can be beneficial for supporting further sugarcane research as a non-destructive criterion in several aspects. This study will help the researcher to uncover the research as a non-destructive criterion in the evaluation of diverse sugarcane cultivars and different growth stages that many researchers were not able to explore. Thus, the alternative high-throughput measurement for further sugarcane research, may be arrived at.

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