



Research Paper

EVALUATION OF COLD TOLERANCE AND PHOTOSYNTHETIC CHARACTERISTICS IN DIFFERENT SUGARCANE GENOTYPES

Shi-Yun Tang^{1, 2}, Yang-Rui Li^{1, 2} and Li-Tao Yang^{1, 2}

¹ Agricultural College, Guangxi University/ State Key Laboratory of Conservation and Utilization of Subtropical Agro-Bioresources, Nanning 530005, China;

² Guangxi Key Laboratory of Sugarcane Genetic Improvement, Sugarcane Research Center, Chinese Academy of Agricultural Sciences, Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530007, China.

Abstract

In order to evaluate the cold tolerance and photosynthetic characteristics in different sugarcane genotypes, and understand the correlations between changes of photosynthetic characteristics and cold tolerance, 7 newly bred sugarcane lines from Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, and 2 commercial main cultivars were used as plant materials in present study. The plants were grown at 4°C chamber for 0, 7, 15, 25, 31 and 38 days as low temperature treatment, while the control was at room temperature (about 25°C). Chilling injury index, chlorophyll content and photosynthetic parameters under low temperature stress were determined. The results showed that chilling injury index increased with the rise of low temperature treatment days, while changes of chilling injury in different sugarcane genotypes were different. Chlorophyll content decreased as the days of low temperature treatment increased. Under cold stress, net photosynthetic rate (Pn) and stomatal conductance (Gs) decreased sharply; Pn and Gs showed significant differences between control and low-temperature treatment. Maximum efficiency of light energy conversion (Fv/Fm), PSII actual quantum efficiency (Φ_{PSII}), reaction center of excitation energy capture (Fv'/Fm'), photochemical quenching coefficient (qP) and electron transfer rate (ETR) significantly decreased under cold stress, but initial fluorescence (Fo), Steady state fluorescence (Fs) and non photochemical quenching coefficient (qNP) increased. Different genotypes showed differences in photosynthetic capacity and chlorophyll fluorescence. Both chlorophyll content and photosynthetic parameters had positive correlations with chilling injury indexes, especially Fv/Fm, Fv'/Fm' and Φ_{PSII} showed highly positive correlation coefficients with the chilling injury indexes, so Fv/Fm, Fv'/Fm', Φ_{PSII} could be used as the indexes of cold tolerance in sugarcane.

Key words: *Sugarcane, Cold Tolerance, Photosynthetic Characteristics, Chlorophyll Fluorescence, Genotype.*

INTRODUCTION

It is well known that sugarcane is one of the sugar crops grown in tropical and subtropical regions, and its growth and development need appropriate temperature. Guangxi is the largest sugarcane producer in China, and its sugar output made up over 70 percent of China's sugar production [1]. Cold temperature affects the growth and development of sugarcane and will lead to considerable decrease in cane yield and sugar quality, especially cold temperature injury, which resulted in a great deal of economic losses in Guangxi in recent years [2] [3] [4] [5] [6]. It has been proved that breeding cold tolerant sugarcane varieties is one of most effective measures to reduce losses caused by cold temperature. Especially, ROC22, the main cultivar in Guangxi, is sensitive to low temperature stress, so it is necessary to breed new cold resistant cultivars.

Chlorophyll fluorescence technique has been applied for years to study the relationship between photosynthesis and environment, which could be used to investigate the efficiency of light energy absorption, status of excitation energy transfer and photochemistry reaction [7] [8] [9]. Chlorophyll fluorescence analysis has been developed as one of the practical techniques; it is fast, sensitive, simple, accurate and scatheless in photosynthesis research, and shows broad prospects for identification of cold tolerance. It was reported that cold tolerance of rice [10] [11], maize [12], wheat [13], banana [14], pepper [15], cauliflower [16], cucumber [17] [18], sugarcane [19], eggplant [20] and tomato [21] could be identified by chlorophyll fluorescence technique.

This study was conducted to investigate the chilling injury index and photosynthetic parameters of different varieties (lines) under cold temperature stress, and reveal the relationship between cold tolerance and change of photosynthetic characteristic, to provide references for screening cold resistant cultivar in sugarcane breeding program.

MATERIALS AND METHODS

Plant materials

Seven newly bred sugarcane lines, namely, GT08-58, GT08-162, GT08-297, GT08-460, GT08-777, GT08-1092, GT08-1180 from Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, and 2 commercial main cultivars, ROC16 and ROC22, were used as plant materials in the present study.

Experimental design

Both 7 sugarcane lines and 2 cultivars used as experiment materials were planted in plastic pots with 25 cm in diameter and 30 cm in depth; each genotype was planted in 6 pots, and 6 single-bud setts were planted in each pot, therefore, 54 pots of seedlings was planted for all treatments. The 9 genotypes were arranged in a completely randomized design with 3 replications, and each replication contained 18 pots. The 116 days old seedlings with about 12 leaves were placed in an artificial climate chamber at 4 °C and subjected to cold treatment; during cold treatment, artificial lights were supplied in the chamber from 8:00 am to 18:00 pm, and the cold treatment was ended after 38 d of treatment.

Measuring method

Chilling injury indexes were measured at 0, 7, 15, 25, and 38 days after cold treatment. According to the damage degree of leaf 0 of the seedlings, chilling injury indexes were divided into 5 grades: The criterion of grade 0 was for those that leaf tip and edge were not curled and leaf was fresh. The criterion of grade 1 was for those that leaf tip was curled but leaf edge was not curled. The criterion of grade 2 was for those that leaf tip and leaf edge were curled. The criterion of grade 3 was for those that leaf tip and edge were curled and leaf tip became withered. The criterion of grade 4 was for those that leaf tip and edge were curled and leaf became withered. The criterion of grade 5 was for those that all leaf became withered and the plant was dying. Chilling injury indexes = $\sum(\text{plant number} \times \text{grade number}) / \text{total number}$.

The sugarcane seedlings were removed out from the chamber and revived for 3 hours at normal temperature, and 3 leaves +2 were taken as samples in each treatment, chlorophyll content was determined with SPAD502, and photosynthesis parameters were measured with

LI-6400 portable photosynthesis system after 30 minutes of dark adaptation, and chlorophyll fluorescence parameters were measured with FMS-2 chlorophyll fluorometer.

Statistical analysis

Analyses of variance (ANOVA) were done with the General Linear Model Procedure of DPS software, and the correlation coefficients between photosynthetic parameters and chilling injury indexes were analyzed.

RESULTS AND ANALYSES

Influence of cold treatment on chilling injury indexes

With the prolongation of low temperature treatment, chilling injury indexes of all the lines (varieties) were increased, but different lines (varieties) had different increasing degree (Table 1). There was no significant difference in chilling injury indexes in different lines at 7 d of low temperature treatment, and only mild cold damage symptoms were appeared at 15 d of low temperature treatment, but at 25 d of low temperature treatment, moderate cold damage symptoms were observed and chilling injury indexes were significantly different in different lines (varieties). At 31 d of low temperature treatment, severe cold damage symptoms appeared, but the difference of chilling injury indexes was not significant in different lines (varieties). At 38 d of low temperature treatment, all plants were dying.

Table 1 The chilling injury index of 9 sugarcane genotypes under low temperature stress

| Treatment Time (d) | Sugarcane genotype | | | | | | | | ROC22 | ROC16 | Mean | Standard deviation |
|-----------------------|--------------------|--------------|--------------|--------------|--------------|---------------|---------------|-----|-------|-------|------|-----------------------|
| | GT08- 158 | GT08- 162 | GT08- 297 | GT08- 460 | GT08- 777 | GT08- 1092 | GT08- 1180 | | | | | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 7 | 0 | 0.5 | 0 | 0.5 | 0.5 | 0 | 0 | 0.5 | 0 | 0.2 | 0.2 | |
| 15 | 0.5 | 1 | 0 | 1 | 1 | 0.5 | 0 | 1 | 0.5 | 0.6 | 0.4 | |
| 25 | 1.5 | 3 | 0.5 | 3 | 3 | 1.5 | 0.5 | 3 | 1.5 | 1.9 | 1.0 | |
| 31 | 3 | 3 | 3.5 | 3 | 3.5 | 4 | 3.3 | 3.5 | 3 | 3.3 | 0.3 | |
| 38 | 4.3 | 5 | 5 | 4 | 4.8 | 4.5 | 4.8 | 4.7 | 4.5 | 4.6 | 0.3 | |

As far as sugarcane genotypes was concerned, GT08-162, GT08-777 and ROC22 suffered from mild cold damage at 7 d of low temperature treatment, which chilling injury indexes were higher at 38 d of low temperature treatment, therefore, GT08-162, GT08-777 and ROC22 were sensitive to low temperature and regarded as cold sensitive varieties. At 15 d of low temperature treatment, GT08-158 and GT08-1092 showed mild cold damage symptom, which chilling injury indexes were lower at 38 d of low temperature treatment, so GT08-158 and GT08-1092 were regarded as cold resistant varieties. GT08-297 and GT08-1180 suffered from mild cold damage at 25 d of low temperature treatment, but their chilling injury indexes were higher at 38 d of low temperature treatment, so GT08-297 and GT08-1180 could only endure cold stress for short time. GT08-460 and ROC16 showed mild symptoms at 7 d of low temperature treatment, but their chilling injury indexes were lower at 38 d of low temperature treatment, so 460 and ROC16 could endure cold stress for a longer time (Table 1).

Influence of cold stress treatment on chlorophyll content and photosynthetic characteristics

With the increasing of low temperature treatment duration, the chlorophyll content was decreased remarkably, and there were significant difference between chlorophyll content at 25 d of treatment and chlorophyll content at 0 d of treatment, but change trend of chlorophyll content differed in different sugarcane genotypes, from 0 to 25 d, GT08-158 had the maximal decreasing range of SPAD, and the order for other genotypes showed GT08-460, ROC16, GT08-162, GT08-297, GT08-1180, GT08-1092, ROC22, GT08-777.

Table 2 Changes of chlorophyll content of 9 sugarcane genotypes under low temperature stress

| Sugarcane genotype | SPAD | | | | Decrease rate (%) |
|--------------------|--------|--------|--------|-------|-------------------|
| | 0 d | 7 d | 15 d | 25 d | |
| GT08-158 | 40.8a | 40.7a | 31.1b | 22.0c | 46.2 |
| GT08-162 | 34.1a | 29.8ab | 26.9b | 26.1b | 23.6 |
| GT08-297 | 42.2a | 39.9a | 37.3ab | 32.8b | 22.2 |
| GT08-460 | 38.5ab | 40.2a | 34.1b | 24.5c | 36.3 |
| GT08-777 | 43.7a | 41.7ab | 40.3ab | 35.8b | 18.3 |
| GT08-1092 | 41.9a | 39.2ab | 36.2ab | 33.5b | 19.9 |
| GT08-1180 | 45.1a | 43.8a | 40.1ab | 35.9b | 20.4 |
| ROC22 | 42.0a | 36.2ab | 32.6b | 34.0b | 19.2 |
| ROC16 | 41.0a | 40.6a | 36.7a | 27.0b | 34.1 |

Notes: The data with different letters are significantly different at $P < 0.05$.

Net photosynthetic rate (Pn) was decreased markedly under low temperature stress, and there were significant differences between Pn at 0 d of treatment and Pn at 7 d of treatment. The decrease from 0 to 25 d was very large, and Pn at 25 d of low temperature treatment was almost zero. For genotypes, GT08-162 had the maximal decrease of Pn and ROC16 had the minimal decrease of Pn.

Under low temperature stress, the decreasing trend of stomatal conductance (Gs) was the same as Pn, and Gs had significant and positive correlation with Pn, so the decrease of Gs should be an important reason for the decrease of Pn. the decrease of Gs at 25 d of low temperature treatment was above 90% of that at 0 day of temperature treatment, Gs at 7, 15 and 25 d of low temperature treatment showed significant difference from Gs at 0 d of low temperature treatment (Tables 2 and 3).

Table 3 Changes of photosynthetic rate and stomatal conductance of 9 sugarcane genotypes under low temperature stress

| Sugarcane genotype | Pn | | | | | Gs | | | | |
|--------------------|---|---|--|--|-------------------|---|---|--|--|-------------------|
| | 0 d ($\mu\text{mol.m}^{-2}.\text{s}^{-1}$) | 7 d ($\mu\text{mol.m}^{-2}.\text{s}^{-1}$) | 15 d ($\mu\text{mol.m}^{-2}.\text{s}^{-1}$) | 25 d ($\mu\text{mol.m}^{-2}.\text{s}^{-1}$) | Increase rate (%) | 0 d ($\text{mmol.m}^{-2}.\text{s}^{-1}$) | 7 d ($\text{mmol.m}^{-2}.\text{s}^{-1}$) | 15 d ($\text{mmol.m}^{-2}.\text{s}^{-1}$) | 25 d ($\text{mmol.m}^{-2}.\text{s}^{-1}$) | Decrease rate (%) |
| GT08-158 | 14.7a | 2.3b | 1.4b | 2.6b | 82.4 | 0 | 7 | 15 | 25 | 95.3 |
| GT08-162 | 24.3a | 3.0b | 1.5b | 0.9b | 96.4 | 0.122a | 0.006b | 0.005b | 0.006b | 94.9 |
| GT08-297 | 24.1a | 4.6b | 3.7b | 1.7b | 93.2 | 0.283a | 0.020b | 0.010b | 0.014b | 96.1 |
| GT08-460 | 23.2a | 3.5b | 2.9b | 2.3b | 90.2 | 0.324a | 0.027b | 0.030b | 0.013b | 95.0 |
| GT08-777 | 18.9a | 4.3b | 1.8b | 3.2b | 83.1 | 0.247a | 0.008b | 0.018b | 0.012b | 97.8 |
| GT08-1092 | 21.6a | 11.0b | 3.0c | 2.1c | 90.1 | 0.186a | 0.042b | 0.004b | 0.004b | 94.8 |
| GT08-1180 | 16.1a | 2.5b | 2.7b | 2.2b | 86.3 | 0.211a | 0.079b | 0.020c | 0.011c | 93.3 |
| ROC22 | 20.5a | 2.0b | 1.0b | 2.8b | 86.2 | 0.161a | 0.005b | 0.005b | 0.011b | 92.9 |
| ROC16 | 21.9a | 6.2b | 4.3b | 4.7b | 78.7 | 0.189a | 0.013b | 0.001b | 0.013b | 94.4 |

Notes: The data with different letters are significantly different at $P < 0.05$.

Table 4 Changes of Fo and Fv/Fm of 9 sugarcane genotypes under low temperature stress

| Sugarcane genotype | Fo | | | | | Fv/Fm | | | | |
|--------------------|------|-------|------|------|-------------------|--------|--------|--------|--------|-------------------|
| | 0 d | 7 d | 15 d | 25 d | Increase rate (%) | 0 d | 7 d | 15 d | 25 d | Decrease rate (%) |
| GT08-158 | 121c | 145bc | 168b | 296a | 144.0 | 0.824a | 0.746b | 0.613c | 0.511d | 37.9 |
| GT08-162 | 106c | 142b | 161b | 346a | 225.7 | 0.818a | 0.695b | 0.442c | 0.338d | 58.6 |
| GT08-297 | 110b | 126b | 122b | 251a | 127.9 | 0.818a | 0.774a | 0.679b | 0.492c | 39.9 |
| GT08-460 | 71c | 103b | 140b | 197a | 177.5 | 0.831a | 0.789a | 0.636b | 0.521c | 37.3 |
| GT08-777 | 101c | 139b | 161b | 247a | 144.6 | 0.813a | 0.774a | 0.669b | 0.463c | 43.1 |
| GT08-1092 | 122c | 142bc | 168b | 213a | 74.6 | 0.814a | 0.765a | 0.630b | 0.559c | 31.4 |
| GT08-1180 | 114c | 124c | 200b | 253a | 121.0 | 0.816a | 0.751b | 0.560c | 0.513c | 37.1 |
| ROC22 | 77c | 147b | 175b | 273a | 254.1 | 0.833a | 0.775b | 0.544c | 0.500c | 40.0 |
| ROC16 | 105c | 128c | 162b | 211a | 100.6 | 0.820a | 0.782a | 0.615b | 0.559c | 31.9 |

Notes: The data with different letters are significantly different at $P < 0.05$.

Influence of low temperature on chlorophyll fluorescence parameters

When the PS_{II} reaction center complex was completely open, the fluorescence value was named initial fluorescence (Fo). Maximal photochemical quantum yield of PSII (Fv/Fm) was used to determine the conversation efficiency of primary light energy. It was reported that under normal conditions the change of Fv/Fm was little, but under stress conditions Fv/Fm would be decreased markedly [22]. From Table 4, it was observed that Fo increased under low temperature, but Fv/Fm decreased, and both Fo and Fv/Fm had significant difference between 25 d of treatment and 0 d of treatment. For genotypes, ROC22 had the largest increase of Fo and the large decrease of Fv/Fm, and GT08-1092 had the lowest increase of Fo and the lowest decrease of Fv/Fm.

From Tables 5 and 6, it was found that actual quantum efficiency (Φ_{PSII}) decreased as the low temperature treatment days increased, and it showed significant difference between 0 d and 25 d treatments. For genotypes, GT08-162 had the highest decrease and GT08-1092 had the lowest decrease of Φ_{PSII} .

Table 5 Changes of Φ_{PSII} and Fv'/Fm' of 9 sugarcane genotypes under low temperature stress

| Sugarcane genotype | Φ_{PSII} | | | | | Fv'/Fm' | | | | |
|--------------------|---------------|--------|--------|--------|---------------|---------|--------|--------|--------|---------------|
| | 0 d | 7 d | 15 d | 25 d | Decrease rate | 0 d | 7d | 15 d | 25 d | Decrease rate |
| GT08-158 | 0.741a | 0.657b | 0.502c | 0.427d | 42.4 | 0.782a | 0.695b | 0.579c | 0.494d | 36.8 |
| GT08-162 | 0.716a | 0.534b | 0.313c | 0.222d | 69.0 | 0.766a | 0.608b | 0.399c | 0.277d | 63.8 |
| GT08-297 | 0.733a | 0.622b | 0.596b | 0.408c | 44.3 | 0.786a | 0.677b | 0.649b | 0.461c | 41.3 |
| GT08-460 | 0.780a | 0.708a | 0.542b | 0.438c | 43.9 | 0.817a | 0.751a | 0.592b | 0.506c | 38.1 |
| GT08-777 | 0.731a | 0.664a | 0.574b | 0.410c | 43.9 | 0.774a | 0.712a | 0.622b | 0.469c | 39.4 |
| GT08-1092 | 0.729a | 0.675a | 0.559b | 0.467c | 35.8 | 0.772a | 0.716a | 0.599b | 0.524c | 32.1 |
| GT08-1180 | 0.722a | 0.633b | 0.436c | 0.413c | 42.8 | 0.775a | 0.691b | 0.506c | 0.469c | 39.5 |
| ROC22 | 0.746a | 0.676a | 0.461b | 0.424b | 43.3 | 0.787a | 0.722a | 0.517b | 0.476b | 39.5 |
| ROC16 | 0.755a | 0.645b | 0.504c | 0.476c | 36.9 | 0.788a | 0.691b | 0.568c | 0.520c | 34.0 |

Notes: The data with different letters are significantly different at $P < 0.05$.

Table 6 Changes of Fs and ETR of 9 sugarcane genotypes under low temperature stress

| Sugarcane genotype | Fs | | | | | ETR | | | | |
|--------------------|-------|-------|-------|------|---------------|---------|--------|--------|--------|---------------|
| | 0 d | 7 d | 15 d | 25 d | Increase rate | 0 d | 7 d | 15 d | 25 d | Decrease rate |
| GT08-158 | 150b | 167b | 183b | 293a | 94.9 | 4.485ab | 3.968b | 4.512a | 3.430c | 23.5 |
| GT08-162 | 140c | 183b | 175bc | 372a | 165.1 | 6.699a | 2.880c | 3.926b | 2.450c | 63.4 |
| GT08-297 | 140b | 160b | 134b | 241a | 72.7 | 6.854a | 5.287b | 3.459c | 3.608c | 47.4 |
| GT08-460 | 86c | 120bc | 153ab | 188a | 118.9 | 5.235a | 4.611b | 5.181a | 3.104c | 40.7 |
| GT08-777 | 127b | 165ab | 180a | 200a | 56.8 | 6.698a | 4.218c | 4.745b | 3.741c | 44.1 |
| GT08-1092 | 159b | 163b | 178ab | 213a | 34.0 | 6.417a | 2.731c | 4.513b | 4.560b | 28.9 |
| GT08-1180 | 149bc | 139c | 186b | 240a | 60.6 | 7.009a | 2.751c | 4.406b | 4.342b | 38.1 |
| ROC22 | 109c | 161b | 179b | 264a | 141.6 | 7.463a | 4.266b | 3.137c | 3.631c | 51.3 |
| ROC16 | 124c | 145bc | 174b | 216a | 74.2 | 5.541a | 3.433c | 4.045b | 4.357b | 21.4 |

Notes: The data with different letters are significantly different at $P < 0.05$.

The maximal photochemical efficiency of PSII in light adaptation status (F_v'/F_m'), steady state fluorescence (Fs) and electron transport rate of PSII (ETR) had the same change trend, which showed decreased as the treatment days increased, and F_v'/F_m' , Fs and ETR had significant difference between 25 d and 0 d treatments. For genotypes, GT08-162 had the highest decrease rate of F_v'/F_m' , Fs and ETR while GT08-1092 had the lowest decrease rate of F_v'/F_m' and Fs. ROC16 had the lowest decrease rate of ETR.

Photochemical quenching coefficient (qP) is used to measure the share of light energy as transported photochemical electron, and it also reflects the open degree of PSII reaction center. Non-photochemical quenching (qNP) was used to measure the proportion of light energy which was dissipated by heat [7] [8].

Data in Table 7 showed that qP decreased under low temperature stress, and it had significant difference between 25 and 0 day treatments. For genotype, GT08-162 showed the highest and ROC16 the lowest qP.

Table 7 Changes of qP, qNP and ETR of 9 sugarcane genotypes under low temperature stress

| Genotypes | qP | | | | | qNP | | | | |
|-----------|--------|---------|---------|--------|---------------|--------|---------|---------|--------|---------------|
| | 0 d | 7 d | 15 d | 25 d | Decrease rate | 0 d | 7 d | 15 d | 25 d | Increase rate |
| GT08-158 | 0.948a | 0.946a | 0.867b | 0.865b | 8.8 | 0.191a | 0.192a | 0.235a | 0.267a | 39.8 |
| GT08-162 | 0.935a | 0.874b | 0.785c | 0.799c | 14.5 | 0.185a | 0.231a | 0.277a | 0.251a | 35.5 |
| GT08-297 | 0.933a | 0.919ab | 0.918ab | 0.884b | 5.2 | 0.163c | 0.355ab | 0.185bc | 0.316a | 94.6 |
| GT08-460 | 0.955a | 0.942a | 0.916a | 0.866b | 9.4 | 0.108b | 0.193ab | 0.219ab | 0.310a | 187.0 |
| GT08-777 | 0.945a | 0.931a | 0.922a | 0.864b | 8.6 | 0.133c | 0.254b | 0.195bc | 0.392a | 193.9 |
| GT08-1092 | 0.945a | 0.942a | 0.933ab | 0.891b | 5.7 | 0.140b | 0.215ab | 0.167ab | 0.282a | 102.0 |
| GT08-1180 | 0.933a | 0.915ab | 0.861bc | 0.879c | 5.7 | 0.170b | 0.300ab | 0.416a | 0.359a | 111.3 |
| ROC22 | 0.949a | 0.935ab | 0.892b | 0.889b | 6.3 | 0.170a | 0.280a | 0.229a | 0.277a | 62.7 |
| ROC16 | 0.958a | 0.931ab | 0.885ab | 0.914b | 4.6 | 0.170b | 0.373a | 0.257ab | 0.222b | 30.5 |

Notes: The data with different letters are significantly different at $P < 0.05$.

It was showed that qNP increased with the duration of cold stress, but the change trend of qNP was different with different genotypes, GT08-777 showed the highest decrease rate and ROC16 showed the lowest decrease rate.

Analysis of correlation coefficients between chilling injury index and photosynthetic parameters

The data in Table 8 showed that SPAD, Pn, Gs and chlorophyll fluorescence parameters had significant correlation with chilling injury index. F_v/F_m , F_v'/F_m' and $\Phi PS II$ had above 0.800 correlation coefficients with chilling injury index, so F_v/F_m , F_v'/F_m' , $\Phi PS II$ could be used as the indexes for cold tolerance of sugarcane genotypes.

DISCUSSION

The effects of low temperature on sugarcane was complicated, and both morphological and physiological characteristics were influenced under cold stress. Low temperature damaged the chloroplast ultrastructure, retarded the chlorophyll biosynthesis, and degraded the photosynthetic pigment, which resulted in a decreased SPAD. In the present experiment, SPAD had significant and negative correlation with chilling injury index, and the result was the same as the result reported by Li et al. [11].

Table 8 correlation coefficients between photosynthetic traits and chilling injury index

| | SPAD | Pn | Gs | Fo | Fv/Fm | Fs | Fv'/Fm' | ΦPS II | qP | qNP | ETR |
|---------|--------|--------|--------|--------|--------|--------|---------|--------|--------|--------|--------|
| Pn | 0.479 | | | | | | | | | | |
| Gs | 0.437 | 0.983 | | | | | | | | | |
| Fo | -0.667 | -0.601 | -0.567 | | | | | | | | |
| Fv/Fm | 0.757 | 0.689 | 0.646 | -0.893 | | | | | | | |
| Fs | -0.661 | -0.539 | -0.498 | 0.965 | -0.819 | | | | | | |
| Fv'/Fm' | 0.748 | 0.728 | 0.684 | -0.889 | 0.989 | -0.836 | | | | | |
| ΦPS II | 0.755 | 0.730 | 0.684 | -0.873 | 0.986 | -0.821 | 0.998 | | | | |
| qP | 0.721 | 0.589 | 0.530 | -0.720 | 0.878 | -0.700 | 0.898 | 0.920 | | | |
| qNP | -0.254 | -0.619 | -0.614 | 0.534 | -0.536 | 0.416 | -0.585 | -0.596 | -0.545 | | |
| ETR | 0.505 | 0.763 | 0.758 | -0.558 | 0.588 | -0.512 | 0.617 | 0.614 | 0.520 | -0.502 | |
| I | -0.700 | -0.655 | -0.619 | 0.792 | -0.872 | 0.703 | -0.846 | -0.833 | -0.649 | 0.419 | -0.471 |

Notes: $P < 0.05 (r = 0.3291)$, $P < 0.01 (r = 0.4238)$

Photosynthesis was the approach of organic material synthesis and energy gain in plant, but photosynthesis was markedly decreased by low temperature stress [10] [12] [14] [17] [20]. In this study, Pn was significantly decreased under low temperature stress, and Pn had a significant correlation with chilling injury index.

Chlorophyll fluorescence analysis represented the intrinsic probe, which was based on chlorophyll in vivo, and was closely related to photosynthesis process. Any influence of low temperature in photosynthesis could be showed by chlorophyll fluorescence analysis, so chlorophyll fluorescence parameters could be used to evaluate the effect of cold stress [19] [23] [24], and help to know where and how it was damage in photosynthetic structure [25]. Under low temperature stress, PS II reaction center would be inactivated with the increase of Fo, and the potential activity of PS II and conversation efficiency of primary light energy would be weakened as ETR, Fv/Fo and Fv/Fm decrease [26].

As one of the sensitive indexes for cold tolerance, Fv/Fm had been used to determine cold tolerance in many plants, such as maize [27], Douglas fir (*Pseudotsuga menziesii*) [28], asparagus bean [29], rice [11]. In many studies, it was reported that the suppression degree of Fv/Fm, Fv/Fo, ΦPSII, Rfd, qP, qN and Yield had significant and positive correlation with the environmental stress degree, but as indexes for stress tolerance, Fv/Fm, Fv/Fo, ΦPSII, Rfd, qP, qN and Yield had different effect in different plant [8]. Li et al. [16] reported that Fv/Fm had significant and positive correlation with cold tolerance in cauliflower. Xie et al. [15] reported that ΦPS II and Fv' had significant and negative correlation with cold tolerance in capsicum. In the present study, with the low temperature treatment prolonging, Fo was increased, and FV/FM, ΦPS II and Fv'/Fm' was decreased. Fo, FV/FM, ΦPS II and Fv'/Fm' had significant correlation with chilling injury index, so Fo, FV/FM, ΦPS II and Fv'/Fm' could be used as the cold tolerance indexes in sugarcane.

Zhang et al. [19] studied the effect of low temperature stress in sugarcane seedlings by chlorophyll fluorescence techniques, and the result showed that Fv/Fm, Fv/Fo, DCPIP, Yield, Rfd, qP and qN were decreased, the primary light energy conversion of PS II and the potential photosynthetic activity were inhibited, and the inhibition degree of cold sensitive varieties significantly higher than that of cold resistant varieties.

In the present study, FV/FM decreased as the days of low temperature increased, and FV/FM had significant correlation with the chilling injury index, and the result was in

accordance with the reports of Zhang et al. [19]. qNP increased under cold stress, which indicated that the non-photochemical dissipation of excessive light energy was enhanced, and it was favorable to protect the photosynthetic apparatus against the damage of cold stress. But the conclusion was inconsistent with the reports of Zhang et al. [19], and further study will be needed to verify the conclusion.

Different sugarcane genotypes had significantly different responses to low temperature. Under low temperature, chlorophyll content, net photosynthetic rate, stomatal conductance, FV/FM, $\Phi PS II$, Fv'/Fm' and qP were decreased, ETR, Fo, Fs and qNP were increased. The chlorophyll content and photosynthetic parameters had significant correlations with the chilling injury index, especially the chilling injury index and Fv/Fm, Fv'/Fm' and $\Phi PS II$ had high correlation coefficients (high than 0.800), which indicated that Fv/Fm, Fv'/Fm' and $\Phi PS II$ could be used as the indexes of cold tolerance in sugarcane. Chlorophyll fluorescence technique could be used for determination of cold stress of sugarcane, which had no damage, stable, fast and accurate characteristics, and it would help sugarcane breeding and improve the efficiency of sugarcane breeding.

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