Evaluation for Photosynthetic Characteristics in upland cotton germplasm based on chlorophyll a fluorescence

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Abstract

Background: The research of photosynthetic characteristics of germplasm resources of cotton (*Gossypium hirsutum* L.) provides reference for genetic improvement on cotton cultivars. Chlorophyll a fluorescence (CF) is widely used for analysis of photosynthetic activity of plants leaves. However, large number of Chlorophyll a fluorescence parameters (CFPs) and inconsistency CFPs application in different studies confuse researchers, which greatly limit the accuracy of photosynthesis analysis. In this study, all 53 CFPs of 189 upland cotton cultivars (strains) measured by handy PEA were screened at 12:00 and 17:00 separately. Results: 9 CFPs including PI(abs), DIo/CSo, PI(abs, total), Sm/T, Vj, Eto/CSo, REo/CSo, RC/CSo and N at 12:00 and 8 CFPs including PI(abs, total), PI(abs), DIo/CSo, Sm/T, REo/CSo, N, Vj and Eto/CSo at 17:00 are suitable for screening of upland cotton cultivars (strains). The screened CFPs classify and evaluate the photosynthetic characteristics of upland cotton cultivars (strains). Conclusions: CFPs selected can be effect and consistent parameters to evaluate photosynthetic activity of cotton germplasm.

Background

Various germplasm resources with genetic diversity are the basis of breeding. The identification of germplasm resources is an important part of breeding work. Photosynthetic product accumulation is the basis of crop growth and development [1, 2], so it is important to evaluate germplasm resources with different photosynthetic activities.

Chlorophyll a fluorescence (CF) has been accepted widely as a reliable and popular tool in photosynthesis research [3, 4]. CF signals contain rich photosynthesis information and can be widely used to quickly monitor state of leaves photosynthesis of plants under stress [5–7] such as temperature [8, 9], moisture [10], salt [11, 12], nutrient element [13, 14], plant senescence [15], mutagenesis [16] and pathogen infection [17, 18] and so on. However, CFPs are strongly affected by the environment [19, 20], which greatly increased difficulties to select cultivars (strains) with good photosynthetic performance. The environmental conditions of one day are variable. It's imperative to detect photosynthetic activity at different times of the day.

Moreover, CFPs are so numerous that most of studies only selected a few [21]. Fv/Fm was used to investigate the effect of nitrogen stress on CFPs of microalgae [22]. The change of photosynthesis under salt stress in rapeseed was investigated with 11 CFPs such as ETo/ABS, Fo, Fv, Fm, Fv/Fm, PI(abs), qP, φEo, RC/ABS, φDo, ψo [23]. The natural aging of leaves was explored using 5 CFPs such as ET/CSo, Fv/Fm, Fo, Fm, and PI(abs) [24]. The selection of limited CFPs for analysis limits the accuracy of interpretation of photosynthetic activity.

In previous studies of photosynthetic properties of plants, CFPs are usually used as an index to detect a few cultivars in environmental stress, leaf aging and plant nutrition and so on [25–28]. Few reports are referred to photosynthetic performance screening of a large number of cultivars (strains), and cotton is no exception. The consistent and representative CFPs used to analyze the photosynthetic properties of cotton remains unknown.

In the present study, 53 CFPs of 189 upland cotton cultivars (strains) (The gramplasms are severed by Institute of Cotton Research of CAAS) were determined at 12:00 (stress) and 17:00 (non-stress) [29, 30] by plant efficiency analyzer. The variation and relationships of CFPs were analyzed, and CFPs selected were used to assess photosynthetic properties of cultivars (strains). The classification and characteristics of 189 upland cotton cultivars (strains) were reviewed. A method for high-throughput analysis of photosynthetic potential of upland cotton germplasms was established.

Methods

Materials

189 upland cotton cultivars (strains), the names of cultivars (strains) are attached to Table S2.

Methods

In 2012, in Anyang, China, during the flowering period of cotton, fluorescence data was measured at 12:00 (suppressed state) and 17:00 (restored state) by a Handy Plant Efficiency Analyser (Handy PEA, Hansatech Instruments Limited, Norfolk, UK) according to Strasser et al [31]. Leaves of upland cotton cultivars (strains) were kept in dark at least for 30 min. Chlorophyll a fluorescence parameters are measured after dark treatment. Repeat is 6 times for each cultivar (strains). The significance of CFPs is shown in the attached (Table 1).

Data analysis

Measured signal were analyzed by Biolyzer 4HP software version 4.0.30.03.02 according to the JIP test. The extracted parameters (Fo, $F_{100\mu s}$, $F_{300\mu s}$, $F_{2ms,}$ $F_{30ms,}$ F_J, $F_{I,}$ Fm.etc.) led to the calculation and derivation of a range of new parameters according to Table S1. Fluorescent data is collected and analyzed using Excel（WPS 2013）and IBM SPSS

Degree of stress (DS) = $(F_{12}-F_{17})/F_{17}$

F_{17} represents the CFPs measured at 17:00. F_{12} represents the CFPs measured at 12:00.

Result

1 Variation of CFPs

The variations of 53 CFPs are determined for the leaves of 189 upland cotton cultivars (strains) at 12:00 and 17:00, respectively. 5 CFPs are not significantly different but 48 CFPs are significantly different between 12:00 and 17:00 (Fig.1; Table 1). This may be due to environmental difference such as temperature, strong light intensity and other stress at 12:00 and 17:00. DS reflects the degree of differences between the CFPs at 12:00 and 17:00. 23 DSs are negative and 25 DSs are positive, indicating significant differences in the DSs of different fluorescence CFPs (Table 1). In order to accurately identify the difference of CFPs of upland cotton cultivars (strains), it is necessary to detect the difference at 12:00 and 17:00.

2 Distribution of CFPs of upland cotton cultivars (strains)

Of 48 CFPs observed in 189 upland cotton cultivars (strains), 23 CFPs at 12:00 tend to lower values distribution than those at 17:00 in the distribution map (Fig. 2). The reduction of these CFPs at 12:00 reflects reduction of photosynthetic function of the leaves. Among the 23 CFPs, 8 CFPs such as Fv/Fm, Fv/Fo, φPo, PI(abs), PI(CSo), PI(CSm), SFI(abs) and D.F. tend to lower value distribution indicating that the photochemical activity of leaves decreased at 12:00 (Fig. 2A-H). 10RC/ABS tends to lower value distribution at 12:00 (Fig. 2I), indicating that coefficient of light energy reaction center decreases. ETo/CSo, ETo/CSm, Kp and SumK tend to lower value distribution (Fig. 2J-M), indicating that the photochemical rate decreased at 12:00. φPo/(1-φPo) and ψo/(1-ψo) tend to lower value distribution(Fig. 2N, O), indicating an increase in the ratio of thermal dissipation to the absorption,capture and electron transfer of light energy at 12: 00; Sm and Sm/T tend to lower value distribution (Fig. 2P, Q), indicating that the plastoquinone library decreases and the reduction rate of plastoquinone library decreases. Both ψo and φEo have a lower value distribution tendency (Fig. 2R, S), indicating that the efficiency of electron transfer was reduced. Fp, Vi, TRo/CSm and RC/CSm tend to lower value distribution (Fig. 2T-W).

Compared with 17:00, a total of 24 CFPs tend to higher value distribution at 12:00 (Fig. 2). The increasing of these CFPs shows reduction of photosynthetic function of the leaves. Fo tends to higher value distribution (Fig. 2X), indicating that energy dissipation increases at 12:00. ABS/RC, ABS/CSo, TRo/CSo, TRo/RC tend to higher value distribution (Fig. 2Y-AC), indicating an increase in the energy absorbed and captured by the unit area reaction center. DIo/RC, DIo/CSo, DIo/CSm tend to higher value distribution at 12:00 (Fig. 2AD-AF), indicating an increase in energy dissipation per unit area and unit reaction center. Fo/Fm and φDo tend to higher value distribution (Fig. 2AG-AH)indicating an increase in the ratio of thermal dissipation to the absorption, capture and electron transfer of light energy. dVG/dTo and dV/dTo tend to higher value distribution (Fig. 2AI,AJ), indicating that the rate of optical reaction center closure increases. REo/CSo and REo/RC tend to higher value distribution (Fig. 2AK, AL), indicating an increase in the electron transfer per unit area and unit reaction center for PSI. φRo, ψRo tends to higher value distribution (Fig. 2AM, AN), indicating that the quantum production used for PSI electron transfer increases. The 8 CFPs such as $F_{100\mu s}$, $F_{300\mu s}$, F_{2ms}, F_{30ms}, Vj, δRo, δRo/(1- δRo) and PI(abs, total) tend to higher value distribution (Fig. 1AO-AV). RC/CSo tends to be distributed at both ends (Fig. 2AW), indicating that some cultivars (strains) have an upward trend and some cultivars (strains) have a downward trend.

Of the 48 CFPs, there are same trends such as Fo, $F_{100\mu s}$, $F_{300\mu s}$, F_{2ms}, F_{30ms}, and ABS/CSo, Kp and SumK, Fo/Fm and φDo at 12:00 and 17:00 (Fig. 2).

3 Screening of CFPs

3.1 Screening of CFPs at 12:00

CFPs with high variation among different cultivars (strains) are suitable for identification of cotton cultivars (strains) with different photosynthetic properties. 38 CFPs were measured at 12:00 such as PI(abs), DIo/CSo, PI(CSm), δRo/(1 − δRo), PI(abs,total), DIo/RC, Sm/T, Kp, PI(CSo), Fo, REo/CSo, SumK, $F_{100\mu s}$, ABS/CSo, DIo/CSm, $F_{300\mu s}$, dVG/dTo, N, SFI(abs), Fv/Fo, φPo/(1-φPo), F_{2ms}, Eto/CSo, Tro/CSo, ψo/(1-ψo), Sm, φDo, Fo/Fm, dV/dTo, REo/RC, D.F. F_{30ms}, ABS/RC, 10RC/ABS, ETo/CSm, Vj, RC/CSo and δRo have a large degree of variation, and the coefficient of variation exceeds 0.1. These CFPs are suitable to screen upland cotton cultivars (strains). 15 CFPs such as Eto/RC, φEo, ρRo , φRo, Fp, Kn, Fm, ABS/CSm, RC/CSm, Tro/RC, ψo, TRo/CSm, Vi, Fv/Fm, Fm and φPo have low variability and coefficient of variation are less than 0.1. These CFPs are not suitable for screening cotton cultivars (strains) (Table 1; Table S3).

Of the 38 CFPs, the correlation coefficient of some CFPs is greater than 0.8, which are determined to be highly correlated. Only one of them was selected in highly related CFPs. Moreover, PI(abs, total)=PI(abs)·δRo/(1−δRo) [32]. So PI(abs, total) and PI(abs) are saved but δRo/(1−δRo) is abandoned. It is finally determined that 9 CFPs such as PI(abs, total), PI(abs), DIo/CSo, Sm/T, N, REo/CSo, Eto/CSo, Vj, RC/CSo are suitable for screening and evaluating the differences of CFPs in upland cotton cultivars (strains) (Table 2).

3.2 Screening of CFPs at 17:00

CFPs with high variation are suitable for identification of cotton cultivars (strains) with different photosynthetic properties. There are large variation coefficients in 41 CFPs measured at 17:00 such as δRo/(1−δRo), DIo/CSo, DIo/RC, dVG/dTo, PI(CSm), PI(abs), PI(abs,total), dV/dTo, PI(CSo), Vj, φDo, Fo/Fm, Sm/T, ψo/(1-ψo), $F_{300\mu s}$, Sm/T, D.F. , ET/CSm, ABS/RC, Fo, Fv/Fo, φPo /(1-φPo), $F_{100\mu s}$, ABS/CSo, DIo/CSm, δRo, φEo, F_{2ms}, Kp, φRo, ψo, RC/CSm, F_{30ms}, ρRo, SumK, Eto/RC, Eto/CSo, REo/CSo, Sm, N, REo/RC. The variation coefficient of these CFPs is more than 0.1 (Table 1, S3). So these CFPs are suitable to select upland cotton cultivars (strains) with different photosynthetic potential. The variation coefficient in the 12 CFPs such as 10RC/ABS, TRo/CSm, TRo/CSo, TRo/RC, Kn, Fv/Fm, φPo, Vi, Fm, ABS/CSm, RC/CSo and Fp is less than 0.1 (Table 1, S3). These CFPs are not suitable for selection of upland cultivars (strains).

Of the 41 CFPs at 17:00, the correlation coefficient of some CFPs is greater than 0.8, which is determined to be highly related (Table 3). Because of high correlation, it is not necessary to list them one by one but select one from high-related CFPs. Finally, it is determined that 8 CFPs such as PI(abs, total), DIo/CSo, Vj, PI(abs), Sm/T, Eto/CSo, REo/CSo and N are suitable for screening upland cotton cultivars (strains).

3.3 Comparison of CFPs obtained by screening at 12:00 and 17:00

The common CFPs obtained at 12:00 and 17:00 include PI(abs,total), PI(abs), DIo/CSo, Eto/CSo, Vj, Sm/T, REo/CSo, N. Compared with 17:00, RC/CSo is unique at 12:00. This result shows that there is no significant difference in the number of response centers per unit area of each strain under the non-stress environment at 17:00. But the values change greatly under the stress environment at 12:00. The results showed that CFPs were greatly affected by environment at 17:00 and 12:00.

4 Upland cotton cultivars (strains) screening

4.1 Upland cotton cultivars (strains) classification and screening

The selected 9 CFPs PI(abs,total), DIo/CSo, Sm/T, N, PI(abs), Eto/CSo, Vj, REo/CSo and RC/CSo are used to classify and review photosynthetic capacity of 189 upland cotton cultivars (strains).

4.1.1 Screening of cultivars (strains) based on PI(abs, total)

PI(abs, total) is the most general parameter in the JIP-test. It allows extending the

research of photosynthetic electron transport activity beyond PS II, involving changes in intersystem electron transport and PS I related processes [33]. According to PI(abs, total) at 17:00, 189 cotton cultivars (strains) are divided into four categories: A, B, C, and D. According to the comparison of the values of PI(abs, total) at 12: 00 and 17: 00 and DSs, the four categories of upland cotton cultivars (strains) A, B, C and D are divided into 12 subcategories(1-12) (Fig. 3A; Table S4).

4.1.2 Screening of cultivars (strains) based on PI(abs)

PI(abs) is a sensitive indicator applied for physiological and environmental screenings, including three functional steps of the photosynthetic activity (light absorption, excitation energy trapping, and conversion of excitation energy to electron transport) [34]. According to the value of PI(abs) at 17:00, 189 cotton cultivars (strains) are divided into four categories: A, B, C and D. Further by the comparison of the values of PI(abs) at 12:00 and 17:00 and DSs, the four categories of upland cotton cultivars (strains) A, B, C and D are divided into 10 subcategories (1-10) (Fig. 3B; Table S5).

4.1.3 Screening of cultivars (strains) based on Eto/CSo

Eto/CSo represents electron transfer per unit area, reflecting the reduction of Q_A re-oxidation at the reaction center [35-37]. In this experiment, Eto/CSo, Eto/RC, and Eto/CSm have a relatively high correlation. Eto/CSo can be used as an important parameter for reflecting photosynthetic electron transfer. According to the value of Eto/CSo at 17:00, 189 upland cotton cultivars (strains) are divided into three categories: A, B, and C. Further, according to the numerical comparison of Eto/CSo and DSs , the three major cotton cultivars (strains) A, B, and C are divided into 9 subcategories (1-9) (Fig. 3C; Table S6).

4.1.4 Screening of cultivars (strains) based on REo/CSo

REo/CSo and REo/RC have a relatively high correlation in this experiment. REo/CSo can be used as an important parameter for reflecting electron transfer at the end of PSⅡ[38]. According to the value of REo/CSo at 17:00, 189 upland cotton cultivars (strains) are divided into three categories: A, B, and C. According to the numerical comparison and DSs of Eto/CSo at 12:00 and 17:00, the three major cotton cultivars (strains) A, B, and C are divided into 6 subcategories (1-6) (Fig. 3D; Table S7).

4.1.5 Screening of cultivars (strains) based on DIo/CSo

DIo/CSo represents thermal dissipation per unit area (at T = 0) [39]. DIo/CSo, DIo/CSm and DIo/CSm have a high correlation. So DIo/CSo can be used as representative CFPs of energy dissipation. According to fluorescence parameter value under non-stress condition at 17:00, 189 cotton cultivars (strains) are divided into three categories: A, B, and C. Further, according to the numerical comparison of DIo/CSo at 12:00 and 17:00 and DSs, the three major upland cotton cultivars (strains) A, B, and C are divided into 11 subcategories (1-11) (Fig. 3E; Table S8).

4.1.6 Screening of cultivars (strains) based on Sm/T

Sm/T expresses the average fraction of opening PSⅡ RCs in the time span from Fo to Fm [40,41]. According to the value of Sm/T under non-stress at 17:00, 189 cotton cultivars (strains) are divided into three categories: A, B, and C. Further, according to Sm/T at 12:00 and 17:00 and DSs, the three major cotton cultivars (strains) A, B, and C are divided into 7 subcategories (1-7) (Fig. 3F; Table S9).

4.1.7 Screening of cultivars (strains) based on N

N represents the number of times of Q_A restored from the time starting illuminating to maximal fluorescence Fm. It is related to the size of the receptor library [42]. According to the value of N non-stress at 17:00, 189 cotton cultivars (strains) are divided into three categories: A, B, and C. Further, according to the value of N at 12:00 and 17:00 and DSs, the three major cotton cultivars (strains) A, B, and C are divided into 11 subcategories (1-11) (Fig. 3G; Table S10).

4.1.8 Screening of cultivars (strains) based on Vj

Vj is the fraction of closed reaction centres (QA^-/QA value) at 2 ms of illumination. Vj is established by the reduction rate of QA to QA^- and the oxidation rate of QA^- to QA [43]. According to the value of Vj non-stress at 17:00, 189 cotton cultivars (strains) are divided into three categories: A, B, and C. Further, according to the value of Vj at 12:00 and 17:00 and DSs, the three major cotton cultivars (strains) A, B, and C are divided into 7 subcategories (1-7) (Fig. 3H; Table S11).

4.1.9 Screening of cultivars (strains) based on RC/CSo

RC/CSo represents the number of unit area reaction centers (at T = 0) [44]. Since the variation coefficient of RC/CSo at 17:00 is small, RC/CSo does not suit to screen cotton varieties, cotton cultivars (strains) are classified using RC/CSo value at 12:00. According to RC/CSo value under 12:00, 189 cotton cultivars (strains) are divided into three categories: A, B, and C. Further, according to RC/CSo numerical comparison and DSs at 12:00 and 17:00, the three major cotton cultivars (strains) A, B, and C are divided into 5 subcategories (1-5) (Fig. 3I; Table S12).

4.2. Review of Typical Cotton cultivars (strains)

According to PI(abs) at 12:00 and 17:00, Ekangmian9, Emian18 and SuQ1 show strong photosynthetic properties. The values of PI(abs) are both high at 12:00 and 17:00, indicating that they have high PS II photosynthetic activity. But PI(abs) values are slightly lower at 12:00 than 17:00, indicating that they are resistant to stress environment at 12:00. The values of RC/CSo, Eto/CSo and N are at a moderate level in 189 cultivars and are slightly lower at 12:00 than 17:00, indicating the number of active reaction centers, the quantum yield of PS II electron transfer and the size of the PSII receptor library are at a moderate level and may be less subject to environmental change. The values of DIo/CSo are low and is slightly lower at 12:00 than 17:00, indicating that the three cultivars (strains) have low energy dissipation. The values of Sm/T are at a moderate level at 17:00 and increased significantly at 12:00, indicating that the ratio of active reaction center to inactivated reaction center is at a moderate level and may increase significantly under stress at 12:00. The value of PI(abs,total) is high, is significantly higher at 12:00 than 17:00, indicating the three cultivars (strains) have PS I photosynthetic performance, which may increase in stress. The values of Vj is low but is higher at 12:00 than 17:00, indicating the ratio of QA^-/QA in the electronic delivery chain at 2ms is low, and may increase under adverse conditions. The REo/CSo value is at a moderate level, with little change from 12:00 to 17:00, indicating the PS I electron transfer efficiency is at a moderate level and may be less affected by the environment. These results show that reduced QA can be oxidized in time and the energy dissipation is low, which are the main reason for the strong photosynthetic performance of Ekangmian9, Emian18 and SuQ1.

According to PI(abs) at 12:00 and 17:00, the photosynthetic performance of Jifeng 908 is at a moderate level in 189 cultivars. PI(abs) are at middle level and are slightly lower at 12:00 than 17:00, indicating PS II photosynthetic performance is at a medium level and reduced under stress. DIo/CSo, PI(abs, total), N, RC/CSo, Eto/CSo, REo/CSo and Vj are in middle level and are higher at 12:00 than 17:00, which show that the dissipated energy, the photosynthetic performance of PS I, the size of receptor bank of PS II, the number of active reaction centers, the electron transfer quantum yield of PS I and PS II, and the ratio of QA^- to QA in the electron transfer chain at 2ms are in moderate and elevated under stress conditions. The high Sm/T values decrease at 12:00 compared to 17:00 indicating that the ratio of the active reaction center to the inactivation reaction center is high and may decrease under stress. These results show that Sm/T may not be the main parameter affecting photosynthetic performance. The electron transfer, the number of active reaction centers, the size of PS II receptor bank and dissipation energy are all at a moderate level. Therefore, Jifeng 908 has moderate photosynthetic properties of PS I and PS II.

According to PI(abs) at 12:00 and 17:00, the photosynthetic performance of Zhongzi 2858 is low. PI(abs) values are low and lower at 12:00 than 17:00, indicating PSII photosynthetic activity is low and reduced due to stress. The values of Eto/CSo, PI(abs, total) and Sm/T are low and is higher at 12:00 than 17:00, indicating that the quantum yield of PS II electron transfer, PS I photosynthetic performance and the ratio of active reaction centres to inactivation reaction centres are low and rise under stress. The high DIo/CSo and RC/CSo values, which are higher at 12:00 than 17:00, indicating high energy dissipation, reaction centres and increase under stress. The N and REo/CSo are at a medium level and are higher at 12:00 than 17:00, indicating that the size of PS II receptor bank and the quantum output of PS I electron transfer are at a medium level and rise under stress. The high Vj values, which are lower at 12:00 than 17:00, show that the ratio of QA^- to QA in electronic delivery chain at 2ms is high and decrease under stress. These results show the low quantum yield of PS II electron transfer, the low proportion of active reaction centres and the high energy dissipation are the main reasons for the low photosynthetic performance.

Discussion

1 The influence of environment on CFPs of upland cotton cultivars (strains)

Environment has a great influence on CFPs of many plants [45,46]. However, no research on effects of environments on variation of CFPs of large numbers of upland cotton cultivars (strains) during a day has been reported. In the present research, significant differences exist in the 48 CFPs of 189 different cultivars (strains) at 12:00 and 17:00 during a day. The distributions at 12:00 are more dispersed than 17:00, which may be related to the different patterns of cultivars (strains) response to stress. The environment changes during a day, so it is necessary to determine the fluorescence CFPs of upland cotton cultivars (strains) at different time in a day.

2 Suitable CFPs for evaluating photosynthetic characteristics of upland cotton cultivars (strains)

How to screen photosynthetic characteristics of a large number of cotton cultivars (strains) is rarely reported. In our study, CFPs are selected by correlation, coefficient of variation and quantitative relations between CFPs at 12:00 and 17:00. Finally, PI(abs), PI(abs,total), DIo/CSo, Sm/T, REo/CSo, N, Vj, RC/CSo and Eto/CSo are selected at 12:00. PI(abs), PI(abs, total), DIo/CSo, Sm/T, REo/CSo, N, Eto/CSo, Vj, and N are selected at 17:00. When evaluating photosynthetic capacity of upland cotton cultivars (strains), PI(abs), PI(abs,total), DIo/CSo, Sm/T, REo/CSo, N, Vj, RC/CSo and Eto/CSo are sufficient. Among these CFPs, PI(abs), ETo/CSo, DIo/CSo, N, and Vj have been widely used [47-49], while REo/CSo, PI(abs, total), RC/CSo, Sm/T are rarely used.

3 Distribution of CFPs

The range of theoretical values for various CFPs is rarely reported. The distribution of CFPs of 189 cultivars (strains) provides breeders with the range of variation of cotton cultivars (strains). Moreover, the distribution of CFPs at 12:00 and 17:00 reveals the changing trend of CFPs of upland cotton when they are subjected to stress compared with non-stress, which provides breeders a reference to the trend of CFPs in the corresponding stress in upland cotton cultivars (strains). Some cultivars (strains) do not conform to the changing trend of CFPs in corresponding stress in upland cotton, which are good research materials for breeders.

4 Significant correlation of CFPs

Correlation between different CFPs is rarely reported in various crops. In the present study, much correlation between many CFPs is found and some correlation is significant. Fo reflects the chlorophyll content of the PS II reaction center [50](Mathur et al., 2011). ABS/CSo reflects the energy absorbed [51]. The correlation coefficient between Fo and ABS/CSo is as high as 0.998 (Table 2, Table 3), indicating that the chlorophyll content is significantly positively correlated with light energy absorption. φPo and φEo represent the maximum yield of primary photochemistry and the maximum yield of electron transfer, respectively [52]. The correlation coefficient between φPo and φEo is as high as 0.962 at 17:00 (Table 3), but the correlation coefficient between φPo and φEo is only 0.775 at 12:00 (Table 2). This shows that the energy captured under non-stress can be used for electron transfer stably, while under stress energy captured used for dissipation increases. PI(abs, total) represents performance of PSI final electron receptor and PI(abs) represents PSII photosynthetic performance index [53]. At 17:00, the correlation coefficient between PI(abs, total) and PI(abs) is 0.94 (Table 3), but at 12:00, the correlation coefficient between PI(abs, total) and PI(abs) is 0.748 (Table 2). It shows that stress of 12:00 has an effect on electron transfer of PS I of leaves of upland cotton.

5 Variation of CFPs

In our study, some interesting variation is found. φPo and Fv/Fm are sensitive CFPs for stress [54-56]. The coefficient of variation of φPo and Fv/Fm at 12:00 and 17:00 is both small (Table 1). It is shown that φPo and Fv/Fm are suitable for detecting extent of stress but not suitable for selecting photosynthetic properties of upland cotton cultivars (strains). RC/CSo reflects the number of response centres per unit area [57]. The variation coefficient of RC/CSo is very small at 17:00 and increased at 12:00 (Table 1), indicating that environmental conditions affect the number of active reaction centers per unit area of leaves.

6 Screening of upland cotton germplasm

In our study, 189 upland cotton cultivars (strains) are classified, which provided photosynthetic traits of germplasm for breeders (Table S4-12). If breeders need to select cultivars (strains) with high photosynthetic potential, which can be obtained in the first category of PI(abs). If breeders need to select cultivars with electronic transfer efficiency, they can select in the first category of ETo/CSo; If breeders need to select cultivars (strains) with good integrated traits, a comprehensive comparison is necessary in categories of CFPs.

Conclusion

A few and consistent CFPs is indispensable to quickly evaluate the photosynthesis of a large number of germplasm. We screen and get 9 CFPs through the coefficient of variation and correlation analysis. Among them, 5 parameters generally recognized and often used in the study of photosynthetic characteristics and 4 parameters rarely used are proved to be suitable for evaluating photosynthetic characteristics of cotton germplasm. Our research immensely promotes the qucik and efficient selection of high light efficiency cotton germplasm and the genetic improvement of photosynthetic cotton germplasm. What further is to screen CFPs of various crops and CFPs of germplasm under different stress. More comprehensive and consistent CFPs of photosynthetic germplasm need to be discovered urgently.

Abbreviations

CF: chlorophyll a fluorescence; CFPs: chlorophyll a fluorescence parameters; DS: degree of stress

Declarations

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Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request

Competing interests

The authors have no conflicts of interest to declare.

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Author's contributions

CMT and XMX designed the project and experiments. YS, WQD, XMD and YHJ. performed the experiments. CMT, XMX, and YS wrote the manuscript. All contributing authors agreed to the final version of the manuscript.

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Tables

Table 1 CFPs of leaves at 12:00 and 17:00 in upland cotton cultivars

CFPs	Mean at 12:00	Standard Error	Coefficient of variation	Mean at 17:00	Standard Error	Coefficient of variation	Average of DSs	Average of absolute DSs	T value	Visibility
Fo	410.105	6.411	0.215	376.05	4.329	0.159	0.092	0.159	6.856	*
Fm	2035.848	11.91	0.080	2056.295	7.712	0.052	-0.005	0.086	-1.205	
$F_{100\ \mu s}$	465.752	7.002	0.207	422.544	4.737	0.155	0.105	0.159	7.896	*
$F_{300\ \mu s}$	521.553	7.725	0.204	469.312	5.227	0.204	0.114	0.164	8.563	*
F_{2ms}	709.827	9.178	0.178	626.576	6.522	0.144	0.138	0.169	11.318	*
F_{30ms}	1089.985	9.907	0.126	967.004	7.769	0.111	0.132	0.149	14.753	*
Fp	1405.266	8.443	0.082	1505.416	5.112	0.047	-0.064	0.085	-10.964	*
Fv/Fm	0.773	0.025	0.045	0.792	0.044	0.057	-0.022	0.044	-6.573	*
Fo/Fm	0.228	0.034	0.152	0.208	0.044	0.215	0.112	0.152	6.574	*
Fv/Fo	3.524	0.046	0.182	4.015	0.046	0.158	-0.111	0.158	-13.439	*
dV/dTo	0.626	0.091	0.149	0.521	0.011	0.301	0.257	0.310	9.176	*
dVG/dTo	0.716	0.141	0.201	0.603	0.213	0.356	0.253	0.317	7.2	*
Vj	0.401	0.043	0.109	0.344	0.077	0.227	0.203	0.252	9.361	*
Vi	0.6	0.035	0.058	0.667	0.036	0.055	-0.098	0.101	-18.653	*
φPo	0.772	0.034	0.045	0.792	0.044	0.057	-0.022	0.044	-6.574	*
ψo	0.599	0.003	0.073	0.656	0.005	0.120	-0.076	0.129	-9.361	*
φEo	0.465	0.044	0.098	0.525	0.075	0.146	-0.099	0.159	-11.188	*
φDo	0.228	0.034	0.152	0.208	0.044	0.215	0.112	0.152	6.574	*
Sm	33.564	0.38	0.156	35.093	0.263	0.104	-0.032	0.155	-3.264	*
N	52.187	0.754	0.198	52.027	0.384	0.103	0.012	0.180	0.345	
Sm/T	0.08	0.002	0.263	0.091	0.001	0.212	-0.042	0.370	-4.103	*
Sum K	2.256	0.034	0.207	2.433	0.019	0.110	-0.078	0.131	-7.693	*
Kn	0.496	0.003	0.080	0.489	0.002	0.057	0.016	0.088	1.577	
Kp	1.760	0.031	0.246	1.943	0.02	0.144	-0.098	0.157	-8.599	*
ABS/RC	2.028	0.18	0.122	1.916	0.023	0.162	0.071	0.117	4.907	*
Tro/RC	1.555	0.009	0.080	1.491	0.006	0.060	0.045	0.072	7.574	*
Eto/RC	0.929	0.007	0.098	0.97	0.008	0.109	-0.032	0.122	-3.83	*
DIo/RC	0.473	0.01	0.286	0.404	0.017	0.378	0.220	0.276	6.164	*
REo/RC	0.622	0.006	0.132	0.494	0.004	0.101	0.264	0.267	19.827	*
REo/CSo	142.867	2.234	0.214	109.135	0.837	0.106	0.306	0.308	16.35	*
RC/CSo	228.035	1.771	0.106	221.394	0.773	0.048	0.030	0.081	4.748	*
ABS/CSo	465.752	7.001	0.207	422.544	4.737	0.155	0.105	0.159	7.896	*
Tro/CSo	355.573	4.222	0.163	329.42	1.706	0.071	0.077	0.126	8.633	*
Eto/CSo	211.842	2.568	0.166	214.08	1.640	0.107	0.000	0.168	-0.596	
DIo/CSo	110.179	40.064	0.365	89.409	33.56	0.382	0.261	0.318	7.601	*
RC/CSm	1017.952	5.87	0.080	1108.655	9.494	0.120	-0.067	0.126	-9.598	*
ABS/CSm	2035.848	11.91	0.080	2056.295	7.712	0.052	-0.005	0.086	-1.205	
TRo/CSm	1570.056	7.161	0.062	1633.752	10.762	0.092	-0.033	0.096	-4.525	*
ETo/CSm	945.863	7.543	0.110	1089.29	12.934	0.166	-0.108	0.194	-9.371	*
DIo/CSm	465.752	7.002	0.207	422.542	4.737	0.155	0.105	0.159	7.896	*
PI(abs)	2.94	0.079	0.372	4.853	0.112	0.322	-0.369	0.403	-21.867	*
SFI(ABS)	2.369	0.032	0.190	2.879	0.039	0.191	-0.163	0.214	-14.619	*
10RC/ABS	5.038	0.044	0.120	5.373	0.037	0.095	-0.059	0.099	-8.639	*
φPo/(1-φPo)	3.524	0.046	0.182	4.015	0.046	0.158	-0.111	0.158	-13.439	*
ψo/(1-ψo)	1.553	0.018	0.158	2.077	0.031	0.206	-0.232	0.267	-16.081	*
PI(CSo)	12568.36	205.581	0.228	19219.78	376.009	0.273	-0.313	0.351	-20.843	*
PI(CSm)	59103.862	1402.277	0.330	101103.932	2389.493	0.329	-0.384	0.410	-21.12	*

D.F.	3.249	0.03	0.129	3.684	0.048	0.184	-0.072	0.204	-9.624	*
PI(abs,total)	5.905	0.123	0.300	5.014	0.113	0.309	-0.816	0.861	6.47	*
φRo	0.309	0.003	0.084	0.266	0.003	0.136	0.180	0.191	14.099	*
ρRo	0.4	0.003	0.087	0.333	0.003	0.110	0.209	0.214	18.653	*
δRo/(1 − δRo)	2.202	0.052	0.324	1.213	0.037	2.335	-0.226	0.295	5.05	*
δRo	0.673	0.005	0.101	0.518	0.006	0.153	0.317	0.327	23.592	*

Annotation: $P_{0.05}$=1.96，df=189. DS = (12CFP-17CFP) / 17CFP. The DS is positive indicating an increase of 12:00 to 17:00, negative indicating a decrease of 12:00 to 17:00, and the values of DS indicates the degree of variation.

Table 2 CFPs correlation analysis at 12:00

Parameter 1	Parameter 2	coefficient of correlation	Parameter 1	Parameter 2	coefficient of correlation
Fv/Fm	Fv/Fo	0.978**	RC/CSo	Eto/CSo	0.927**
Fv/Fo	Fo/Fm	-0.978**	Vj	dV/dTo	0.853**
Fv/Fm	Fo/Fm	-1**	DIo/CSo	Fo	0.972**
ABS/RC	ABS/CSo	0.925**	DIo/CSo	$F_{100µs}$	0.972**
ABS/CSo	ABS/CSm	0.835**	DIo/CSo	$F_{300µs}$	0.97**
TRo/RC	TRo/CSo	0.839**	DIo/CSo	F_{2ms}	0.954**
TRo/RC	TRo/CSm	0.384**	DIo/CSo	F_{30ms}	0.91**
TRo/CSo	TRo/CSm	0.401**	DIo/CSo	DIo/RC	0.991**
Eto/RC	Eto/CSo	0.8**	DIo/CSo	DIo/CSm	0.958**
Eto/RC	ETo/CSm	0.752**	DIo/CSo	φDo	0.986**
Eto/CSo	ETo/CSm	0.585**	φEo	DIo/CSo	-0.94**
DIo/RC	DIo/CSo	0.981**	TRo/CSo	DIo/CSo	0.745**
DIo/RC	DIo/CSm	0.929**	Fv/Fo	DIo/CSo	-0.914**
DIo/CSo	DIo/CSm	0.972**	DIo/CSo	ABS/CSo	0.972**
Fv/Fm	φPo	1**	DIo/CSo	ABS/RC	0.93**
φPo	φEo	0.775**	DIo/CSo	Fv/Fm	-0.987**
φEo	φDo	-0.775**	DIo/CSo	φPo	-0.986**
ψo	φEo	0.925**	DIo/CSo	φPo/(1-φPo)	-0.953**
Sm	N	0.918**	Vj	ψo	-1**
Sm	Sm/T	-0.539**	Vj	dV/dTo	0.853**
N	Sm/T	-0.738**	dV/dTo	dVG/dTo	0.962**
Kn	Kp	0.839**	Vj	dVG/dTo	0.702**
Sum K	Kp	0.999**	Vj	φEo	-0.925**
Sum K	Kn	0.863**	PI(abs)	SFI(ABS)	0.976**
REo/RC	REo/CSo	0.933**	PI(abs)	10RC/ABS	0.865**
RC/CSo	RC/CSm	-0.146**	PI(abs)	PI(CSo)	0.87**
φPo	φPo/(1-φPo)	0.978**	PI(CSo)	PI(CSm)	0.946**
ψo	ψo/(1-ψo)	0.975**	PI(abs)	PI(CSm)	0.976**
φRo	ρRo	0.871**	PI(abs)	D.F.	0.938**
ρRo	δRo/(1 − δRo)	0.417**	PI(abs)	PI(abs,total)	0.748**
δRo	δRo/(1 − δRo)	0.706**	Fp	Fm	0.903**
Fo	ABS/CSo	0.998**	PI(abs)	sumK	0.838**
Fo	ABS/RC	0.914**	PI(abs)	Kp	0.855**
Fv/Fo	φDo	-0.978**	Eto/RC	Eto/CSo	0.8**
Fv/Fo	DIo/CSo	-0.953**	DIo/CSo	dVG/dTo	0.875**
TRo/CSo	DIo/CSo	-0.919**	ϕRo	Fv/Fm	0.235**
DIo/CSo	φPo/(1-φPo)	-0.953**	ϕRo	dVG/dTo	-0.507**
PI(abs)	DIo/CSo	-0.821**	DIo/CSo	φRo	-0.188**
PI(abs)	Fo	-0.804**	Vj	ρRo	0.207**

Table 3 CFPs correlation analysis at 17:00

Parameter 1	Parameter 2	coefficient of correlation	Parameter 1	Parameter 2	coefficient of correlation
Fv/Fm	Fv/Fo	0.944**	DIo/CSo	φPo/(1-φPo)	-0.953**
Fv/Fo	Fo/Fm	-0.944**	DIo/CSo	Fo	0.959**
Fv/Fm	Fo/Fm	-1**	DIo/CSo	$F_{100µs}$	0.958**
dV/dTo	dVG/dTo	0.99**	DIo/CSo	$F_{300µs}$	0.953**
Vi	Vj	0.803**	DIo/CSo	F_{2ms}	0.931**
Sm	Vj	-0.538**	DIo/CSo	F_{30ms}	0.882**
Sm	Vi	-0.74**	Fp	Fm	0.619**
ABS/RC	ABS/CSo	0.937**	DIo/CSo	DIo/RC	0.991**
ABS/RC	ABS/CSm	-0.552**	DIo/CSo	DIo/CSm	0.958**
ABS/CSo	ABS/CSm	-0.447**	DIo/CSo	φDo	0.986**
TRo/RC	TRo/CSo	0.743**	φEo	DIo/CSo	-0.94**
TRo/RC	TRo/CSm	-0.569**	TRo/CSo	DIo/CSo	0.745**
TRo/CSo	TRo/CSm	-0.451**	Fv/Fo	DIo/CSo	-0.914**
Eto/RC	Eto/CSo	0.908**	DIo/CSo	ABS/CSo	0.958**
Eto/RC	ETo/CSm	0.884**	DIo/CSo	ABS/RC	0.948**
Eto/CSo	ETo/CSm	0.853**	DIo/CSo	Fv/Fm	-0.986**
Fv/Fm	φPo	1**	DIo/CSo	φPo	-0.986**
φPo	φEo	0.962**	DIo/CSo	φPo/(1-φPo)	-0.914**
φEo	φDo	-0.962**	Vj	ψo	-1**
φPo	ψo	0.949**	Vj	dV/dTo	0.984**
ψo	φEo	0.995**	dV/dTo	dVG/dTo	0.99**
ψo	φDo	-0.949**	Vj	dVG/dTo	0.956**
Sm	N	0.837**	Vj	φEo	-0.995**
Sm	Sm/T	0.059	PI(abs)	SFI(ABS)	0.954**
N	Sm/T	0.113**	PI(abs)	10RC/ABS	0.888**
Kn	Kp	-0.489**	PI(abs)	PI(CSo)	0.979**
Sum K	Kp	0.996**	PI(CSo)	PI(CSm)	0.989**
Sum K	Kn	-0.408**	PI(abs)	PI(CSm)	0.993**
REo/RC	REo/CSo	0.901**	PI(abs)	D.F.	0.868**
RC/CSo	RC/CSm	0.057**	PI(abs)	PI(abs,total)	0.94**
φPo	φPo/(1-φPo)	0.944**	Fp	Fm	0.619**
ψo	ψo/(1-ψo)	0.955**	PI(abs)	sumK	0.903**
φPo/(1-φPo)	ψo/(1-ψo)	0.918**	PI(abs)	Kp	0.919**
PI(abs)	PI(abs,total)	0.94**	Eto/RC	Eto/CSo	0.908**
ϕRo = REo/ABS	ρRo = REo/TRo	0.966**	DIo/CSo	dVG/dTo	0.964**
Fv/Fo	φDo	-0.944**	φRo	Fv/Fm	0.835**
φEo	Fv/Fo	0.946**	φRo	dVG/dTo	-0.865**
RC/CSo	Eto/CSo	0.719**	DIo/CSo	φRo	-0.801**
φRo	ψo	0.803**	Vj	ρRo	-0.803**
ρRo	ψo	0.803**	Fo	ABS/CSo	0.998**
Vj	dVG/dTo	0.956**			

Figures

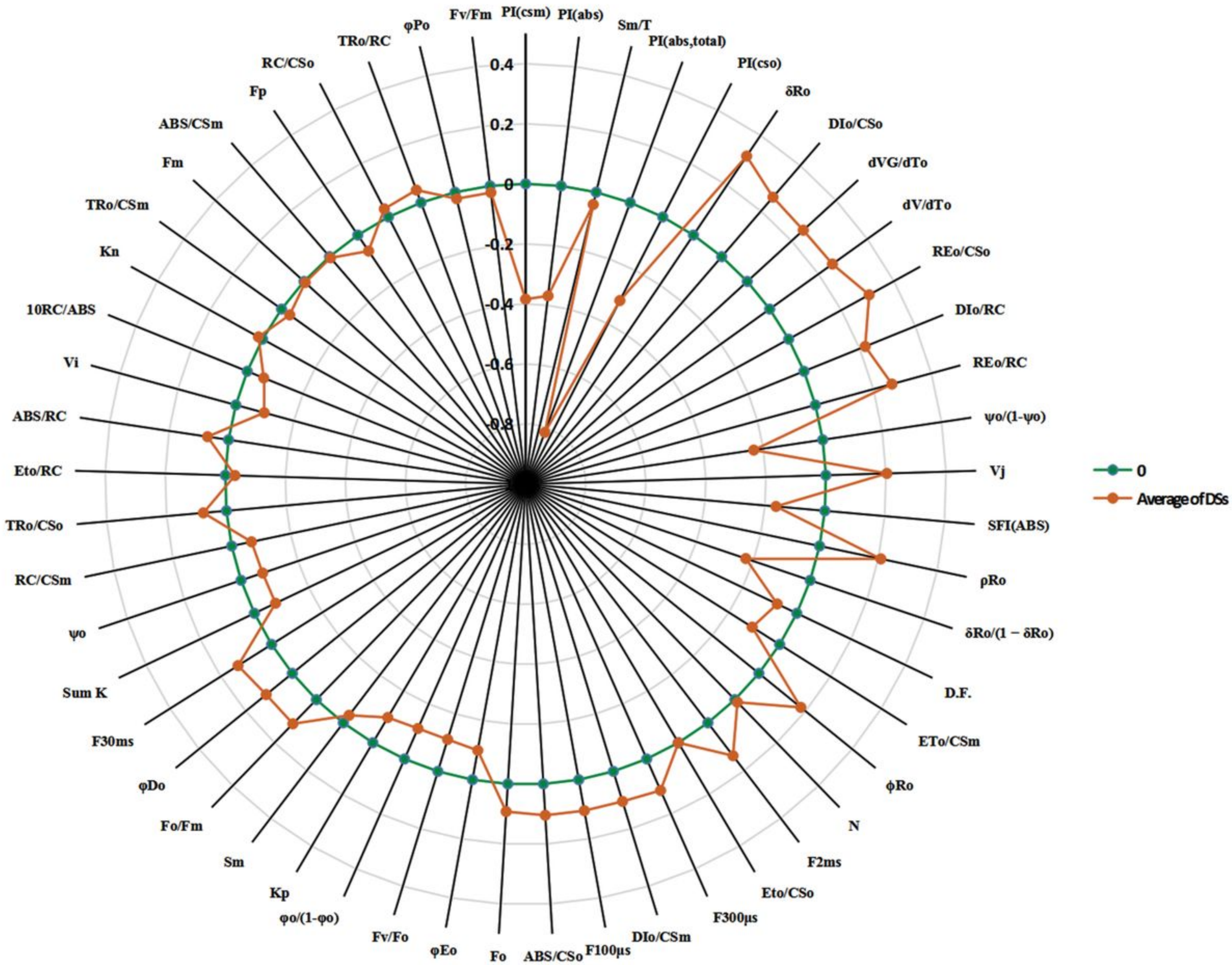

Figure 1

The comparison of CFPs at 12:00 and 17:00

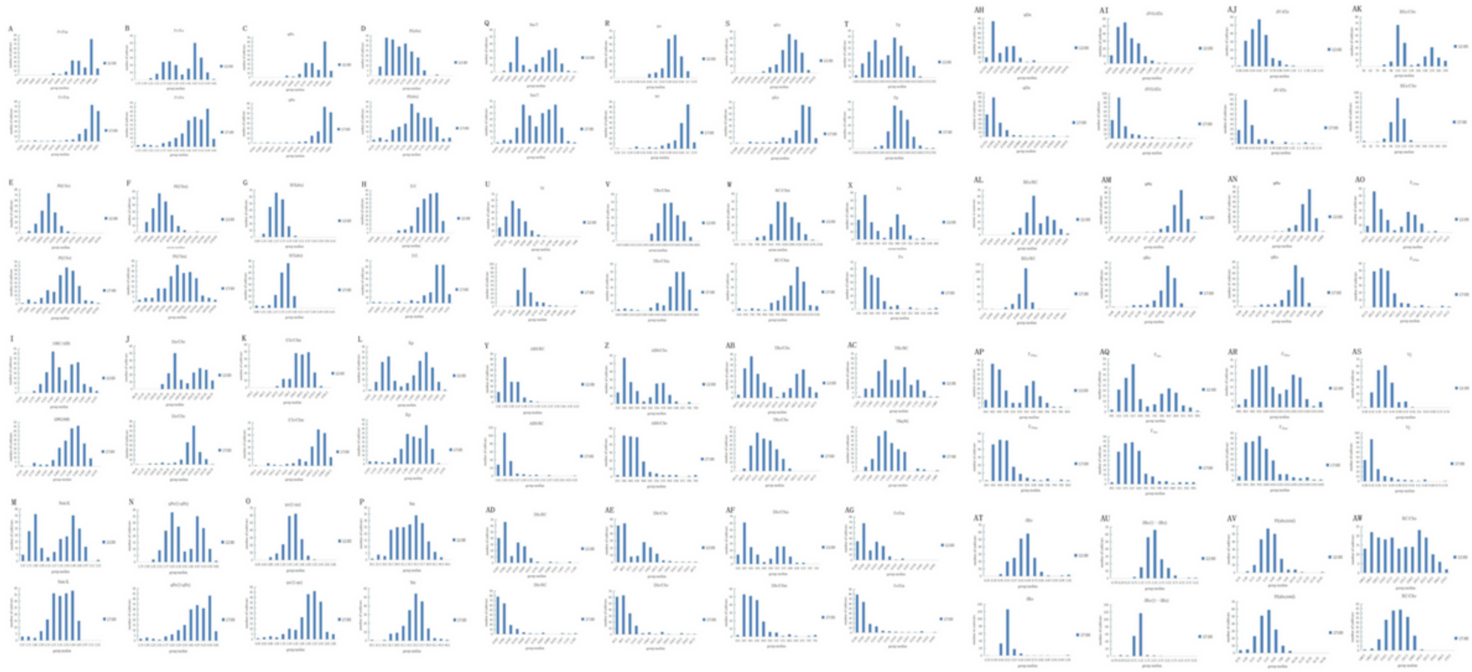

Figure 2

The distribution of CFPs at 12:00 and 17:00

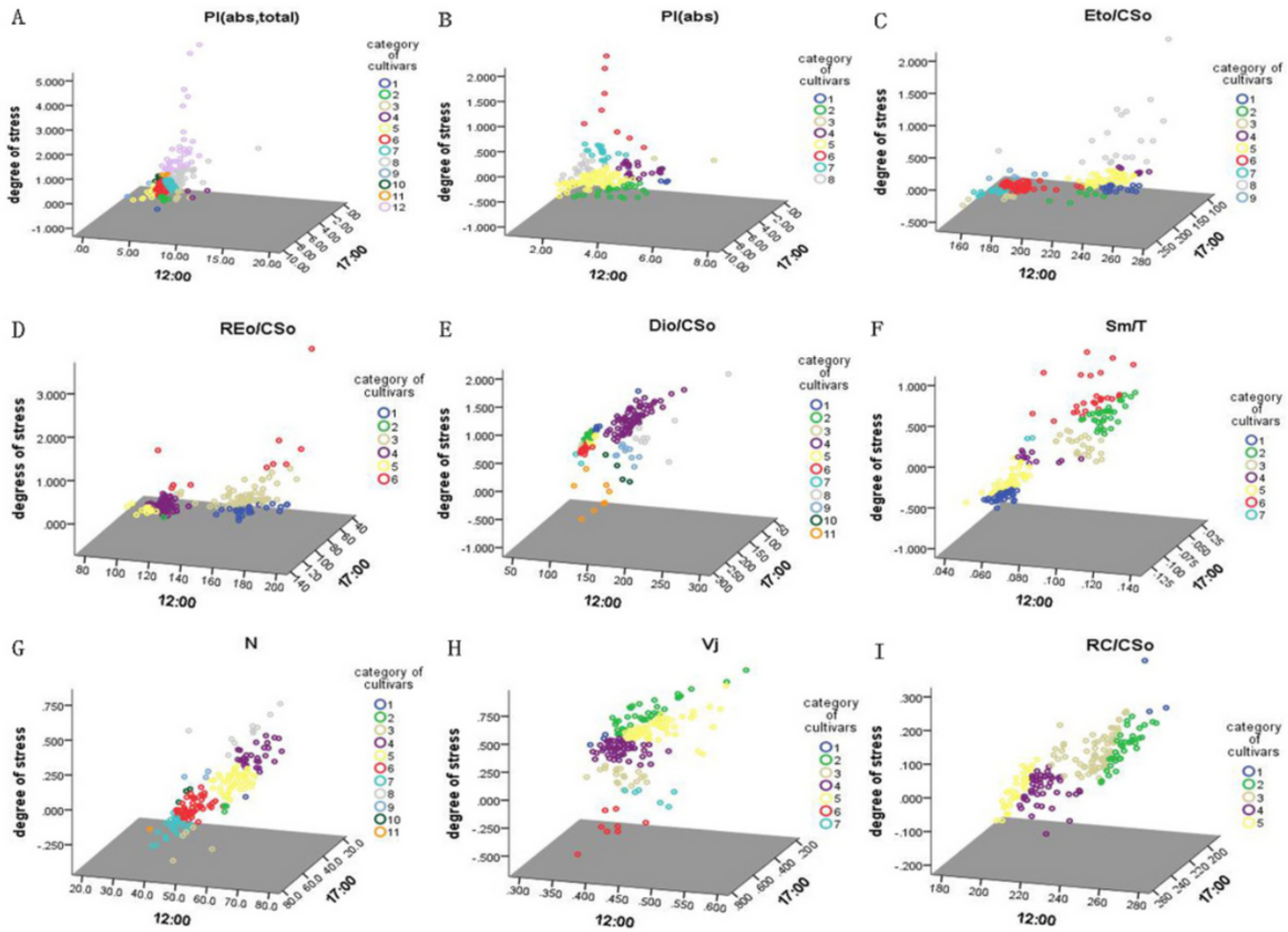

Figure 3

The classification of cultivars by 9 CFPs

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTables.docx