Analysis of Differences in Wheat Infected with Powdery Mildew Based on Fluorescence Imaging System

Shizhou Du[1,2], Qinhong Liao[2,3(✉)], Chengfu Cao[1], Yuqiang Qiao[1], Wei Li[1], Xiangqian Zhang[1], Huan Chen[1], and Zhu Zhao[1]

[1] Anhui Academy of Agricultural Sciences, Hefei 230031, China
dsz315@sina.com, caocfu@126.com, yuqiangqiao@163.com, jtlw2007@163.com, xiangqian111@163.com, chenhuanyeah@163.com, zhaozhu0114@aliyun.com

[2] National Engineering Research Center for Information Technology in Agriculture, Beijing 100097, China
67076566@qq.com

[3] College of Life Science and Forestry, Chongqing University of Art and Science, Chongqing 402160, China

Abstract. This study aimed to investigate the variation characteristics of rapid light-response curves of wheat leaves infected with powdery mildew. According to the heterogeneity between two selection patterns of area of interest (AOI), determination of fluorescence induction parameters and fitting of rapid light-response curves were conducted based on fluorescence imaging system in wheat powdery mildew experimental plots. The results showed that relative electron transport rate rETR was reduced with the increase of disease severity level; rETR of the rectangle selection pattern was relatively low. Specifically, the reduction in rETR is mainly influenced by the decrease of absorption coefficient Abs. Among fitting parameters of rapid light-response curves, the potential and the maximum relative electron transport rate, initial slope, light suppression parameter and semi-saturation intensity were reduced with the increase of disease severity level; the heterogeneity of fitting parameters between two selection patterns reflected the “critical state” of leaf fluorescence characteristics. Infected leaves at severe level (80 %) had relatively low light-harvesting capacity and tolerance to strong light, which easily caused light inhibition. According to the lateral heterogeneity analysis of photosynthesis of wheat leaves infected with powdery mildew, there was relatively high heterogeneity between fluorescence parameters of wheat infected leaves, especially in leaves with lesions on the surface.

Keywords: Fluorescence imaging system · Powdery mildew · Rapid light-response curve · Difference

1 Introduction

Wheat powdery mildew (*Blumeria graminis* f. sp. tritici) is a major disease in worldwide wheat production, which is caused by infection of *Erysiphe qraminis* D.C. f. sp. tritici marchal. After infection of *Erysiphe qraminis* D.C. f. sp. tritici marchal., greatly

D. Li and Z. Li (Eds.): CCTA 2015, Part II, IFIP AICT 479, pp. 68–75, 2016.
DOI: 10.1007/978-3-319-48354-2_7

propagated pathogenic bacteria disrupt the moisture transport in wheat leaves and cause the destruction of chlorophyll. With the disease progression, wheat leaves show yellow spots or patchy lesions under severe situations, powder mildew layer is attached to the surface, leading to leaf chlorosis and yellowing or even death [1, 2].

Modulated pulse fluorescence analyzer (Imaging-Pam Mini-version: 24 × 32 mm) has a relatively large detection window to acquire fluorescence parameters within a range of 24 × 32 mm [3, 4]. In addition, fluorescence imaging system can clearly distinguish leaf lesion area and non-lesion area due to its visual advantage. Powdery mildew lesion areas on wheat leaves are covered by conidiospores of pathogenic bacteria. Due to the differences in the thickness and status of covering layer, chlorophyll fluorescence characteristics of the lesion areas are relatively complex [5]. Currently, few studies have been reported on the advantages of fluorescence imaging system combining with disease stress. Therefore, in this study, two selection methods of area of interest (AOI) were adopted as data sources which respectively represent the conventional optical fiber probe mode and visualization window mode [6], characteristics of the fluorescence differences in rapid light-response curve between non-lesion area and mixing area of wheat leaves infected with powdery mildew were investigated, to reflect the "critical state" of fluorescence characteristics of infected leaves and explore the dynamic changes in light response of wheat leaves under stress of different levels of powdery mildew, which provided theoretical basis for the in-depth research of fluorescence characteristics of wheat diseases.

2 Experiments and Methods

2.1 Design of Field Trials

During March–June 2013, the experiment was carried out in National Precision Agriculture Experimental Base of Xiaotangshan Town, Changping District, Beijing City, China. Wheat variety Jingshuang 16 was selected as experimental material, which is highly susceptible to powdery mildew. A total of three treatments were designed. After pathogen inoculation on April 5, 2013, wheat powdery mildew was controlled using Triazolone with effective amounts of 240, 120 and 30 g/hm^2 at booting stage (April 30, 2013) to develop different incidence levels; infected wheat leaves in control group were treated with water. The planting density in experimental plots was 3 000 000 seedlings/hm^2, with a row spacing of 0.2 m. The rest of the managements were in accordance with the field practices.

2.2 Leaf Selection

Wheat heading stage (May 10, 2013) is the outbreak period of wheat powdery mildew in experimental plots. Wheat leaves infected with different incidence levels of powdery mildew (top second leaf) in various experimental plots were collected as experimental samples. Based on the percentage of the coverage area of lesion hyphal layers on infected leaves accounting for the total area of leaves, disease severity levels (SL) of wheat leaves were classified into four levels, including mild level (10 %), moderate level (50 %), severe level (80 %) and control level (0 %, with no symptom in the whole plant), respectively. Ten leaves at each level were collected.

2.3 Fluorescence Data Acquisition

Based on the pulse amplitude modulation (PAM) techniques, a modulated pulse fluorescence analyzer (Imaging-Pam Mini-version: 24 × 32 mm, Heinz Walz GmbH, Effeltrich, Germany) was used to detect the wheat leaves with disease severity levels according to the chlorophyll fluorescence induction kinetic curve and rapid light response curve [7].

2.4 Rapid Light-Response Curve Fitting

PS II relative electron transport rate was calculated in accordance with the formula: rETR = (Fm′−F) /Fm′·PAR·0.5 Abs. Specifically, PAR indicated the photosynthetic active radiation; 0.5 was the approximate PS II distribution coefficient of light energy; Abs indicated the absorption coefficient. Rapid light-response curves (RLCs) are variation curves of relative electronic transport rate (rETR) with photosynthetic active radiation (PAR). Rapid light-response curve was fit with the least square method using SPSS19.0 software and drawn using Origin 8.0 software.

2.5 Fluorescence Parameter Selection

Modulated pulse fluorescence analyzer has the advantage of image visualization, which provides a variety of data selection patterns of area of interest (AOI). Therefore, lesion areas on wheat leaves infected with powdery mildew could be clearly distinguished. In this study, four sample points were selected in the non-lesion area based on a circle pattern, with a radius of 1 mm, detected values of the four sample points were averaged; four points were selected in the mixing area based on a rectangle pattern, with the length and width of 8 × 28 mm.

3 Results and Discussion

3.1 Changes in Rapid Light-Response Curves of Wheat Leaves Infected with Powdery Mildew Based on Different Selection Patterns

The relative electron transport rate rETR based on different selection patterns rapidly increased with the increase of photosynthetic active radiation PAR, while rETR increased slowly and tended to decline when PAR reached 600 − 1 000 μmol/m^{2}s. As shown in Fig. 1, the control (0 %) remained relatively high relative electron transport rate rETR; with the increase of severity level, rETR was gradually reduced. Differences in rETR between two selection patterns were gradually enhanced with the increase of severity level, indicating the negative response of the expansion of leaf lesion area to rETR value. At the same severity level, the relative electron transport rate based on a rectangle pattern was lower; for instance, at severe level (80 %), rETR based on a rectangle pattern was only a half of control (0 %).

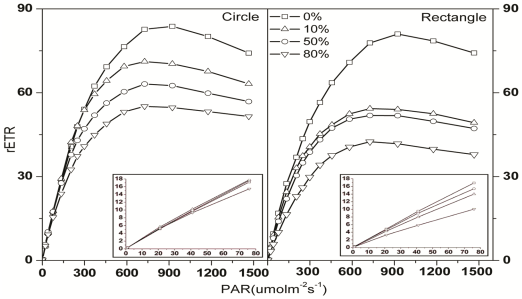

Fig. 1. Rapid light-response curves of wheat leaves infected with powdery mildew based on different selection patterns (left: selected based on a circle pattern; right: selected based on a rectangle pattern)

3.2 Changes in Characteristic Parameters of Rapid Light-Response Curves

As shown in Fig. 2, fitting parameters Pm, α, β and Ik were constantly reduced with the increase of severity level, which were all higher with a circle sampling pattern in non-lesion area; changes in fitting parameters with the disease severity level slightly varied between two selection patterns.

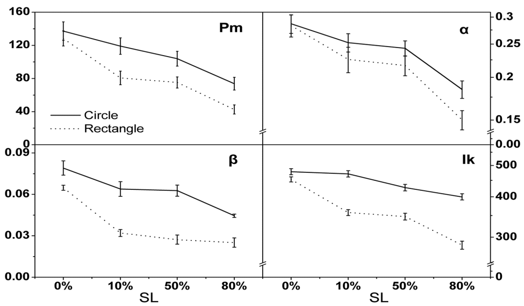

Fig. 2. Changes in characteristic parameters of rapid light-response curves

Variation coefficients of the maximum potential relative electron transport rate Pm in non-lesion area and mixing area were 24.7 % and 43.2 %, respectively. Pm in mixing area at severe level (80 %) was only 33.1 % of normal leaves. Initial slope α reflected the level of light-harvesting capacity of leaves, and the variation coefficients of two selection patterns were 17.6 % and 24.9 %, respectively. Light suppression parameter β reflected the slope of the decline curve, and the variation coefficients of two selection patterns were 22.6 % and 43.8 %, respectively. After powdery mildew infection, β value in mixing area declined greatly and varied slightly between infected leaves, suggesting that the dynamic regulation ability of PS II reaction center under strong light was reduced

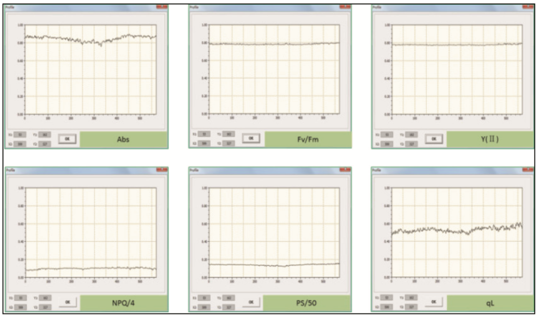

Fig. 3. Heterogeneity of imaging fluorescence parameters of healthy wheat leaves (linear)

after infection, resulting in insufficient capacity of heat dissipation of excess light energy. Semi-saturation light intensity Ik reflected the capacity of leaves to bear strong light, and the variation coefficients of two selection patterns were 8.4 % and 19.4 %, respectively. Ik value in mixing area varied slightly between mild level (10 %) and moderate level (50 %); Ik value was greatly reduced at severe level (80 %), indicating low light-harvesting capacity of leaves and relatively low tolerance to strong light, which could easily cause light inhibition or even light damage.

3.3 Heterogeneity of Chlorophyll Fluorescence Imaging at Different Severity Levels

Conventional fluorescence modulation equipments mostly use optical fiber as signal conductor, such as PAM-2100/2500 and Mini-PAM, which can only be adopted to detect partial photosynthetic activity of leaves. Different parts of leaves have different tissue structures and chlorophyll contents. Therefore, fluorescence characteristics in different parts of the same leaf have lateral heterogeneity, but using fiber optic probes to obtain fluorescence characteristics of a point is difficult to reflect photosynthetic characteristics of an entire leaf. Based on plant chlorophyll fluorescence imaging techniques, the lateral heterogeneity of photosynthesis of wheat leaves infected with powdery mildew was analyzed, which was represented by variation coefficient (CV). Fluorescence imaging monitoring system can even applied to detect the invisible damages in early period, thus revealing the stress state and demonstrating the damage mechanisms.

In healthy wheat leaves (Fig. 3), Abs of leaf veins was slightly reduced, while other fluorescence parameters showed relatively high consistency, with variation coefficients lower than 2 %, suggesting the homogeneity of healthy wheat leaves.

Fluorescence parameters of wheat leaves infected with powdery mildew (SL = 80 %) showed significant heterogeneity. To be specific, fluorescence parameters Fv/Fm (CV = 5.3 %) and PS/50 (CV = 7.8 %) had relatively low heterogeneity; the variation coefficients of other fluorescence parameters Abs, Y(II), NPQ/4 and qL were 9.32 %, 15.12 %, 21.37 % and 25.43 %, respectively. As shown in Fig. 4, fluorescence parameters Fv/Fm, Y(II), PS/50, qL and Abs has consistent variation trends, while NPQ/4 had a contrary variation trend with other fluorescence parameters, which indicated that there was relatively high heterogeneity between fluorescence parameters of wheat leaves infected with powdery mildew, especially in leaves with lesions on the surface. Relative electron transport rate rETR (expressed as PS) is an expression way of photosynthetic rate. A large number of studies have shown that rETR has a linear relationship with photosynthetic oxygen evolution rate or CO2 assimilation rate before light saturation. Under the same light conditions, rETR is mainly affected by leaf absorbance Abs and PS II actual quantum yield Y(II). Y(II) of wheat leaves infected with powdery mildew had relatively great variation coefficients, suggesting that rETR changes were greatly influenced by Y(II).

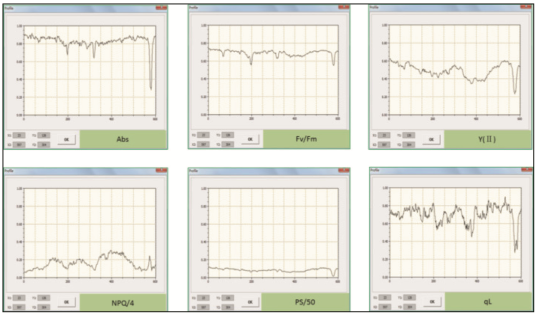

Fig. 4. Heterogeneity of imaging fluorescence parameters of wheat leaves infected with powdery mildew (linear)

Heterogeneity of imaging fluorescence parameters of plant leaves infected with wheat powdery mildew pathogen was mainly produced by the combined action of powdery mildew cleistothecium on leaf surface and damaged photosynthetic systems. Fluorescence techniques provide lossless probes for revealing plant physiology, and the fluorescence imaging system can accurately characterize the physiological characteristics of plant leaves infected with wheat powdery mildew pathogen.

4 Conclusions

In this study, based on the fluorescence imaging system, two selection patterns were adopted to characterize the heterogeneity of rapid light-response curves of wheat leaves infected with powdery mildew, thereby approximately reflecting the "critical state" of fluorescence characteristics of leaves. Rapid light-response curves of non-lesion area of infected leaves vary due to the different severity levels, suggesting that there are differences in PS II system between different severity levels, which may be related with the blocked PS II electron transport caused by the destruction of moisture and chloroplast in leaves, thus leading to the reductions in actual photochemical quantum yield Y (II) and relative electron transport rate rETR [10]. The rectangle selection pattern of wheat leaves infected with powdery mildew covers lesion area and non-lesion area, and the physical damage and inhibition degree become serious, leading to differences in fitting parameters of two selection patterns, which is consistent with the characteristics of other crops under disease stress [11]. Fluorescence parameters of all the pixels in the line (with width of 3) of infected leaves at moderate level (50 %) were shown in Fig. 5. The results showed that: under the same light intensity, the actual photochemical quantum yield Y(II) of leaves is reduced with the increase of photosynthetic active radiation PAR, especially in the lesion area.

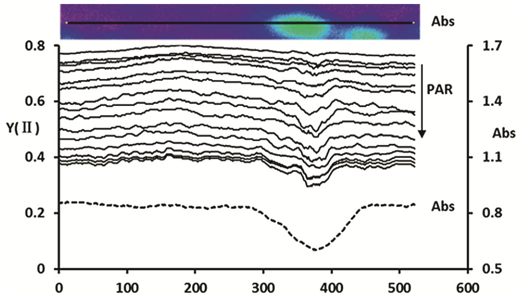

Fig. 5. Heterogeneity analysis of infected leaves based on fluorescence parameters Y (II) and Abs (SL = 50 %)

Relative electron transport rate rETR is an expression way of photosynthetic rate. Results confirm that rETR has an ideal linear relationship with photosynthetic oxygen evolution rate or CO_2 assimilation rate before light saturation. During the detection process, the light adaptation time under different PAR gradients is very short (10 s), resulting in low interference effects on the natural photosynthetic state of samples [12]. Under the same light intensity, rETR is mainly affect by actual quantum yield Y(II) and absorption coefficient Abs. The heterogeneity of Y(II) (CV = 1.45 %-6.37 %) and Abs (CV = 9.17 %) under different light conditions is shown in Fig. 5, which suggests that absorption coefficient Abs of wheat leaves infected with powdery mildew has relatively great effects on rETR, reflecting the heterogeneity of rETR between two selection patterns, which is mainly due to the differences in red band absorption ability of powdery mildew spores on leaf surface and mesophyll cells, and this conclusion has been verified by leaf spectrum detection [13].

Acknowledgements. Funds for this research was provided by Outstanding Youth Fund of President of Anhui Academy of Agricultural Sciences (13B0203); National Science and Technology Support Program of China (2011BAD16B06, 2012BAD04B09).

References

1. Morgounov, A., Tufan, H.A., Sharma, R., et al.: Global incidence of wheat rusts and powdery mildew during 1969–2010 and durability of resistance of winter wheat variety Bezostaya 1. Eur. J. Plant Pathol. **132**(3), 323–340 (2012)
2. Liu, L.Y., Song, X.Y., Li, C.J., et al.: Monitoring and evaluation of the diseases of and yield winter wheat from multi-temporal remotely-sensed data. Trans. Chin. Soc. Agric. Eng. **25**(1), 137–143 (2009)

3. Pedro, S., María, J.Q.: Assessment of photosynthesis tolerance to herbicides, heat and high illumination by fluorescence imaging. Open Plant Sci. J. **3**, 7–13 (2009)
4. Pedro, S., María, J.Q.: Assessing photosynthesis by fluorescence imaging. J. Biol. Educ. **45**(4), 251–254 (2011)
5. Elisa, G., Angeles, C.: Applications of chlorophyll fluorescence imaging technique in horticultural research: a review. Sci. Hortic. **138**(1), 24–35 (2012)
6. Lucia, G., Sauro, M., Elena, D.: Effects of ozone exposure or fungal pathogen on white lupin leaves as determined by imaging of chlorophyll a fluorescence. Plant Physiol. Biochem. **45**(10–11), 851–857 (2007)
7. Olaf, K., Jan, F.H.S.: The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth. Res. **25**(3), 147–150 (1990)
8. Schreiber, U., Gademann, R., Ralph, P.J., et al.: Assessment of photosynthetic performance of prochloron in lissoclinum patella in hospite by chlorophyll fluorescence measurements. Plant Cell Physiol. **38**(8), 945–951 (1997)
9. Platt, T., Gallegos, C.L., Harrison, W.G.: Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. J. Mar. Res. **38**(4), 687–701 (1980)
10. Kuckenberg, J., Tartachnyk, I., Noga, G.: Detection and differentiation of nitrogen-deficiency, powdery mildew and leaf rust at wheat leaf and canopy level by laser-induced chlorophyll fluorescence. Biosyst. Eng. **103**(2), 121–128 (2009)
11. Stephen, A.R., Julie, D.S.: Chlorophyll fluorescence imaging of plant-pathogen interactions. Protoplasma **247**(3–4), 163–175 (2010)
12. Deng, P.Y., Liu, W., Han, B.P., et al.: A comparative study in photosynthesis heterogeneity of Viola baoshanensis and V. yedoensis. Acta Ecol. Sin. **27**(7), 2983–2989 (2007)
13. Huang, W.J., David, W.L., Niu, Z., et al.: Identification of yellow rust in wheat using in-situ spectral reflectance measurements and airborne hyperspectral imaging. Precis. Agric. **8**(4–5), 187–197 (2007)