A Method for Obtaining Field Wheat Freezing Injury Phenotype Based on RGB Camera and Software Control

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Keywords: RGB imaging, Wheat freezing injury, Freezing injury phenotype, Vegetation coverage, Frost damage grade

Posted Date: August 17th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-779098/v1>

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Version of Record: A version of this preprint was published at Plant Methods on November 26th, 2021. See the published version at <https://doi.org/10.1186/s13007-021-00821-7>.

Abstract

Low temperature freezing stress has adverse effects on wheat seedling growth and final yield. The traditional method to evaluate the wheat injury caused by the freezing stress is by visual observations, which is time-consuming and laborious. Therefore, to effectively and efficiently quantify the wheat freezing injury in the field environments, a high-throughput phenotyping system was developed in this paper, namely, RGB FREEZING INJURY SYSTEM. The system is able to automatically collect, processing, and analyze the wheat images collected using a mobile phenotype cabin in the field conditions. A data management system was also developed to store and manage the original images and the calculated phenotypic data in the system. A group of 128 wheat varieties were planted with replicates under a freezing environment. Canopy images of the wheat were collected at the seedling stage and three image features were extracted for each wheat samples, including ExG, ExR and ExV. The results show that the developed methods can clearly distinguish wheat samples with different wheat freezing injury scores. The automatic phenotypic analysis method of freezing injury provides a solution for high-throughput phenotypic analysis of field wheat and can quantify the stress caused by freezing injury at the seedling stage. The method has a certain guiding significance for wheat breeding.

Introduction

Wheat yield level has a direct impact on national food security, because wheat is a main food crop (Wei, Zhang, Shi, Wang, Chen, Song, & Tao, 2015). Low temperature freezing injury is one of the main natural problems affecting wheat growth (Sofalian, Mohammadi, Aharizad, Moghaddam, & Shakiba, 2006), and it occurs in the seedling stage, which results in losses in the agricultural production (Skinner & Mackey, 2009; Kolesnichenko, Pobezhimova, Grabelnych, Tourchaninova, Korzun, & Koroleva, 2003). Slight freezing injury leads to different degrees of yield reduction, whereas severe freezing injury will lead to low yield and serious losses in agricultural production (Bjarko, 1988; Wang, Wang, Wang, Wang, & Yin, 2011). Therefore, the detection of freezing injury is necessary for agricultural breeding and guidance. Gao et al (2006) and Ye et al (2009) determined the effects of low temperature stress on crops by judging the changes of chemical elements in seedlings. However, these methods are time-consuming and laborious; they hinder promotion and application. Therefore, for food security, a rapid and non-destructive diagnostic method of wheat low temperature stress is needed to accelerate the breeding of excellent frost resistance wheat genotypes (Wu, Zhu, Cheng, Ma, & Wang, 2012; Cobb, Declerck, Greenberg, Clark, & McCouch, 2013). Optical method has advantages in rapid nondestructive testing. Therefore, many researchers have tried various optical sensors to evaluate crop growth (Bussemeyer, Lucas, Mentrup, Daniel, Möller, Kim, Erik, Alheit, & Katharina, 2013; Zhou, Mou, Zhou, Zhou, Heng, & Nguyen, 2021; Bodner, Alsalem, Nakhforoosh, Arnold, & Leitner, 2017). In terms of freezing damage detection, Li, Chen, Yang, and Zhang (2006) conducted a preliminary study on chlorophyll fluorescence characteristics of cotton seed seedlings under low temperature stress. Wu, Zhu, Cheng, Ma, and Wang (2012) studied the degree of freezing damage of wheat seedlings in multiple periods by using imaging spectrum and image integration technology, which can accurately reflect the parts of wheat seedlings with freezing damage.

For the large-scale measurement of freezing injury, remote sensing can potentially measure freezing injury (Feng, Yang, Cao, & Ding, 2009). Remote sensing monitoring was used to assess the freezing injury of spring wheat, summer maize (Zhang, Chen, Su, & Zhou, 2001), and winter wheat (Zhang, Chen, Zheng, Zou, Chen, & Fu, 2006) in Ningxia. To systematically evaluate the severity of winter wheat freezing injury, Wang et al. (Wang, Guo, Wang, Huang, Xiao-He, & Dong, 2013) proposed a grey system model (GSM) to monitor the extent and distribution of winter wheat freezing injury. However, the studies used too-low spatial resolution of remote sensing data; thus, the monitoring effect is not satisfactory. Truss-type phenotypic equipment is widely used for the complex field environment; it has the advantages of high acquisition accuracy, good stability, high imaging quality, and all-day plant monitoring (Virlet, Sabermanesh, Sadeghitehran, & Hawkesford, 2017; White, Andrade-Sanchez, Gore, Bronson, Coffelt, & Conley, 2012).

The field mobile phenotype cabin is a large-scale phenotype platform that acts on the field environment to realize the functions of real-time climate simulation, full coverage of crop varieties, and remote real-time tracking simulation. The device is based on the RGB FREEZING INJURY SYSTEM, which is composed of a mobile warehouse in the field and an RGB camera. It is used to realize the automatic and efficient acquisition and identification of wheat canopy freezing injury images. In addition, the software of data acquisition control and phenotype extraction was specifically developed to facilitate shooting and operation. In this paper, the RGB images of different wheat genotypes obtained from the phenotypic warehouse in the field were used to analyze and calculate the wheat seedling stage vegetation coverage. Through image processing technology, the freezing injury part of winter wheat was analyzed and identified, and the freezing injury level was divided to provide reference information for wheat breeding and agricultural activities.

Materials And Methods

Experimental setup

The winter wheat experiment was conducted in Baima base of Jiangsu Province, China (31°36' 36" N, 119° 10' 48" E). The region has a mid-latitude subtropical monsoon climate with simultaneous hot and rainy seasons. The annual precipitation of Nanjing is more than 1000 mm, thereby making it a humid area. The annual average temperature of the whole province is between 13.6 °C and 16.1 °C. A soil nitrogen content of 24.8 kg·ha⁻¹ was measured before fertilization. Three nitrogen levels were set up, and the fertilization rates were as follows: N1, 0 kg·ha⁻¹; N2, 180 kg·ha⁻¹; and N3, 240 kg·ha⁻¹ (urea, 46% N). All plots were irrigated a month before sowing and a month after sowing. A total of 128 wheat varieties were evaluated under freezing. They were planted with six replicates in two-row plots of 1.5m in length with 0.5 m row spacing, resulting in a total of 768 plots in the test field. The experimental materials were planted on Nov. 11, 2020 with a planting density of 390 plants per square meter. A repeat group was set up for each variety. The field moving phenotypic bin and wheat partition are shown in Fig. 1.

The wheat plants experienced severe freezing stress at the seedling stage. From Jan. 6, 2021, the cold air affected Jiangsu from south to north, resulting in the continuous drop of temperature. From the 6th to the 7th, the cooling range of Baima base was more than 10 °C, and the lowest temperature fell below - 9 °C from the 6th to the 8th. This low temperature had a very wide range of influence and resulted in serious freezing in the whole province, leading to the freezing injury of winter wheat. On Jan. 9, 2021, the wheat freezing injury were rated by an experienced breeder rated on a scale of .

Hardware component of the phenotyping platform

The overall structure of the phenotyping platform is shown in Fig. 2. The platform consists of a top connection structure (part-a), a 2D rotating mechanical structure (part-b), and an imaging box (part-c). The top connection adopts a long bolt (A1) to connect the upper connecting plate (A3) and the bottom connecting plate (A2), which are connected to the field mobile warehouse. In the 2D rotating mechanical structure, the head of the imaging box is connected by fixed axis (B2), rotated by rotating axis (B1), and locked by B3 to ensure an 120° imaging angle. An industrial computer (C), in the imaging box receives instructions from the network to control the camera to take photos and sends back the download address of each photos. C1, C2, and C3 are connected to the power supply, the camera, and the computer, respectively. A industrial RGB camera (D4, Model: MV-CA050-20GC, brand, manufacturer location) is fixed on the camera fixed bracket (D1) and connected to the fastening pulley (D2) fixed on the D3 slide rail. This rail can allow the adjustment of the vertical height to realize the adjustment of the camera shooting distance. In this study, the camera was positioned to take images at a nadir view at 1.5 m above the ground. Detailed parameters of the camera can be found in Table S1.

The overall shell of the machine body is made of an aluminum profile, which is lightweight but has high strength and hardness. A power supply (Uninterruptible Power Supply) is used to adjust the voltage sensitivity, detect the abnormal line voltage, and automatically switch the battery operation to protect the connected equipment.

Software components

The hardware system needed to be equipped with a development software. The software acquisition and analysis system required Visual Studio 2019 to write the interface and MATLAB to write the analysis algorithm. The capture control of the image was performed. Fine tuning of the view was done through the image return, thereby obtaining the device information and achieving the best size and parameter configuration. Moreover, the RGB image was captured and displayed. The captured image was automatically saved to the specified folder, and the processed binary image was also saved to the specified address for further calculation and research.

Image processing

Figure 4 shows the image processing flow, which includes the following four steps. (A) Image acquisition of wheat canopy freezing injury. The top view of crop canopy was obtained by hanging the device at a suitable height. The height of the image acquisition should be consistent. Three RGB images were taken

for each nitrogen content gradient and wheat species plot, and a total of 2304 pictures were taken. The time of image shooting was selected under actual natural light in the field from 12 pm to 3 pm, and the light intensity of the whole shooting process was 2750–2850 LX. (B) The obtained image was preprocessed first in the image segmentation. Then, the binary image was segmented, the contour was extracted, and the region of interest was obtained. (C) Image feature extraction was performed through binary segmentation of the image. The target region pixel and related color features were obtained. (D) The canopy structure parameters were calculated, and the degree of freezing injury of wheat was obtained.

Image segmentation

For the collected images of wheat freezing injury, the values of R, G, and B of green plants and background soil had different characteristics (Hamuda, Glavin, & Jones, 2016). By separating the original image into three independent primary color planes and then selecting different color feature combinations, each pixel in the image was converted to enhance the contrast between the target crop and the background soil in the image. The extraction method of over green index minus over red index satisfactorily identified the green plants in the image; suppressed the shadow, withered grass, and soil background; and highlighted the green plants in the image. The segmentation process is shown in Fig. 5. First, the Otsu(2007) method was used to automatically select the threshold value of gray data. Then, the gray value of the super green minus the super red component of each pixel was calculated and compared with the threshold value. According to the comparison result, the pixel was divided into plants or background. The color feature values to be extracted included excessive green (ExG) (Meyer, & Neto, 2008), excessive red (ExR) (Mao, Wang, & Wang, 2003), and EXV (Motohka, Nasahara, Oguma, & Tsuchida, 2010).

$$ExG = 2g - r - b \quad (1-1)$$

$$ExR = 1.4r - g \quad (1-2)$$

$$ExV = ExG - ExR = 2g - r - b - 1.4r + g \quad (1-3)$$

In the above formula, r, g, and b are the normalized pixel values of the red (R), green (G), and blue (B) channels, i.e., $r = R/(R + G + B)$, $g = G/(R + G + B)$, and $b = B/(R + G + B)$, respectively.

The binarized image was obtained by threshold segmentation, and then, the ROI contours containing all the plants were extracted. The cv2.bitwise_and function was called in OpenCV, and the mask was applied on the binary data (binarized picture), retain the white area (plant) image pixels of the mask image, and

remove the black area (background) image pixels to complete the image segmentation by removing the background.

Image feature extraction

Vegetation coverage (VC) is an important indicator to measure the change of vegetation growth status on the land surface. It usually refers to the percentage of the total area of vegetation (leaves, stems, and branches) projected vertically on the ground to the total area of the statistical area. VC reflects the photosynthesis capacity of plants and the growth status of plants (Cheng, Zhang, & Chen, 2008). When using this system to collect canopy vertical angle image, we should try to avoid using the widest or farthest shot end of the lens focal length and use a smaller aperture to reduce the geometric distortion of the image. After image processing, the background of wheat image was removed, and the proportion of green pixels of wheat to the total pixels was calculated by using the segmented gray image, i.e., the proportion of wheat vertical projection area to the total area. The result was the wheat coverage of the study area. Considering that the coverage detected was the proportion of the green part, and the wheat freezing injury resulted in shrinkage, the bud tip was yellow and white. Thus, the area of the freezing injury part was not included in the coverage range. The coverage can reflect the wheat growth after freezing injury and can effectively characterize the degree of wheat freezing injury.

Statistical analysis

Statistical analysis was conducted using the SPSS statistics packages (IBM SPSS statistics 26, Inc., Chicago, IL, USA). A two-way analysis of variance (ANOVA) was to evaluate the effects of nitrogen treatments (N1, N2 and N3 as fixed factors) on the vegetation coverage, leaf area, green index and withered leaf area of wheat varieties (random factors).

ANOVA analyses were also conducted to see if there is significant differences in the image features among the wheat freezing injury rates. To determine the coverage relationship between the cold resistant and non-freeze resistant genotypes, the mean values were compared with the mean values of Least-SignificantDifference ($P < 0.05$).

Results And Discussion

Performance analysis of wheat freezing injury

The cause analysis of wheat bud tip yellowing was as follows. In the wheat seedling stage, if the plant lacked nutrients, then it turned yellow. The most common condition was the lack of nitrogen, phosphorus, and potassium. The lack of nitrogen will result in small and weak seedlings, few and weak tillers, and the leaves of lose their green and yellow colors. In serious cases, the chlorophyll will decrease or disappear, resulting in the withering of leaf tips and the yellowing and withering of the lower part of old leaves. The effect of nitrogen deficiency was excluded, because wheat growth was better without nitrogen treatment. The lack of phosphorus leads to the decrease of secondary roots, low number of tillers, dark green leaves, and yellow leaf tips. Potassium deficiency generally starts from the tip of the old leaves and then extends

downward along the leaf vein. Later, seedlings fall to the ground, which was not consistent with the symptoms of wheat in this experiment; thus, it was excluded. Unreasonable mechanical modulation may lead to too-deep sowing due to the use of broadcast sowing method, resulting in too-long underground stem growth. When wheat seedlings are unearthed, seeds show high nutrient consumption, which would lead to slender leaves, poor root development, and thin and yellow seedlings (Tanveer, Imtiaz, Sohail, Kissana, & Ab Bas, 2003).

The main performance of wheat in this experiment was as follows. Before cooling, the wheat seedling growth was normal. After exposure to low temperature, the leaves turned yellow. The windward side showed more serious damage, and the leaves showed symptoms of deformation and water loss. After being blown by the wind and exposed to the sun, leaves became dry white or yellow white. The leaves were folded, bent, and then dried up gradually after solar irradiation, which was consistent with the performance of a plant that has freezing injury (Sofalian, Mohammadi, Aharizad, Moghaddam, & Shakiba, 2006). Thus, freezing injury was the main research variable.

Differentiation of freezing injury degree by vegetation coverage

The figure shows the coverage levels of 128 genotypes of winter wheat under the conditions of no nitrogen application, low nitrogen application, and high nitrogen application. In general, in the comparison of wheat subjected to treatment with three nitrogen levels after freezing injury, wheat growth in the high-nitrogen fertilizer plot was not good; in 87.4% of the varieties, high nitrogen treatment led to the lowest coverage. The wheat that did not receive nitrogen fertilizer ($24.8 \text{ kg} \cdot \text{ha}^{-1}$ nitrogen concentration) grew better than the others. Among 62.2% of the varieties, the wheat with no nitrogen fertilizer showed the best coverage. Figure 7 shows that the coverage of the experimental plot was distributed in the range of 0–0.20. The highest vegetation coverage rate was 0.21, and the lowest was 0.02. The coverage difference between various species was obvious, which reflected the degree of resistance of different species to freezing injury.

According to the evaluation standard of wheat freezing injury (Table S2), 128 kinds of field wheat were classified. The differences between the image factors and the degree of freezing injury were analyzed by variance analysis. Significant differences exist in the average vegetation coverage of 128 wheat varieties at the budding stage, and different nitrogen treatments had significant effects on the frost resistance of wheat, especially the significant correlation between the coverage and the evaluation of frost damage (Table S3). Figure 10 presents a box chart of vegetation coverage and wheat frost damage classification, which showed that the coverage demonstrated a good distinction between plants with frost damage and those without frost damage. Therefore, the coverage was selected as the main division segment in this paper. Based on the coverage, different genotypes of wheat were classified and graded. The four intervals were as follows: < 0.05 (severe freeze injury area); $0.05\text{--}0.10$ (severe freeze injury area); $0.10\text{--}0.15$ (slight freeze injury area); and > 0.15 (no obvious freeze injury area). The physical images of different levels and the segmented images are shown in Fig. 8. Based on this method, the frost damage grades of 128

genotypes and three nitrogen treatments were determined. The four intervals corresponded to 1, 2, 3, and 4 grades. Grade 1 represented the lightest injury, and grade 4 represented the most serious. The growth of different genotypes of wheat in extreme cold weather can be clearly seen through the field plot rating screening presented in Fig. 9. The excellent genotypes (grade 1 freezing injury) calibrated by this method included Huaimai 33, Zhoumai 16, and Luomai 26. Further investigation showed that these wheat plants had strong cold resistance, resisted low temperature, and belonged to varieties with satisfactory cold resistance. Similarly, through the screening of the rating method, it was found that the cold resistance of wheat varieties with higher freezing injury level was generally weak. Therefore, this method can provide a scientific basis for the screening of wheat varieties with freezing injury resistance; it can also provide agricultural production guidance, reduce the dead seedling rate, and improve the yield.

Severe freezing injury area

The coverage can be used to distinguish the areas with obvious difference in frost damage degree., as shown in Fig. 11 (a). However, the specific situation of freezing injury in a low coverage area was different. A large number of leaves withered and turned yellow, as shown in Fig. 11 (b). The lack of seedlings in a ridge field was caused by various factors, as shown in Fig. 11 (c). When the wheat coverage was more than 0.10, the wheat in the experimental plot grew better, and there was no obvious lack of seedlings. When the wheat coverage was in the range of 0.0–0.10, the wheat growth in the experimental plot was not good, and there was an obvious lack of seedlings. Therefore, we inferred that the low wheat coverage may have been caused by dead seedlings (Wang, Xiao, Liang, Wang, Wu, &Chen, 2018). In the low coverage area, there were 63 experimental plots with seedling deficiency compared with the standard wheat without seedling deficiency; the seedling deficiency rate was > 0%. Among them, seven experimental plots belong to non-nitrogen treatment; 23 experimental plots belonged to low nitrogen treatment; and the remaining 33 experimental plots belonged to high nitrogen treatment. The corresponding coverage was low, indicating that the lack of seedlings greatly affected the coverage, and freezing injury mostly occurred in the high nitrogen fertilizer range.

The freezing injury is not serious

A significant correlation existed between the coverage of most wheat genotypes and frost resistance, but there were some special cases. In this paper, the following representative genotypes of wheat were selected to study the correlation of their coverage. Although the vegetation coverage varied among genotypes in the frost resistant group, the vegetation coverage of non-frost resistant genotypes was either significantly lower than or not significantly different from that of frost resistant genotypes (Fig. 12). Thus, the system could only distinguish the obvious frost damage.

Through field observation, freezing damage was found to also exists in the plot with high coverage, as shown in Fig. 13 (a). The yellow area in the figure showed freezing damage. The leaf tips withered and became yellow and white. The high coverage plot grew well. Thus, the frost damage area was small, and the injury was concentrated on the leaf tip. Compared with the overlapping situation, the low coverage

plot better identified the frost damage area by color. In this experiment, the image was converted to HSI channel to extract the threshold color, and the withered leaves of high coverage area were extracted, as shown in Fig. 13 (b) (the pseudo color image) and Fig. 13 (c) (withered leaves extraction). We found that this extraction method had some deviations, such as sunlight reflection, similar background color, and yellowing of bud color, but it was still able to reflect a certain degree of freezing injury.

Conclusion

This paper discussed the possibility of using the micro phenotype information acquisition platform with RGB camera and remote control as a mobile phenotype analysis technology. In this experiment, automatic image segmentation and character extraction were used to promote the study of plant phenotypic information. The RGB to HSV color space and color threshold extraction can effectively remove the image background. At the same time, binarization is used to calculate the leaf area coverage rate, seedling shortage rate, and other characteristics

(1) The basic software and hardware system selection of phenotype acquisition equipment, including the design of the structure and control system, and a software system for shooting calculation, was developed for this equipment. This equipment can be used for camera control shooting and image preliminary analysis.

(2) To further explain the operation principle of the system, the operation process and data acquisition principle are interpreted one by one, including image processing process, segmentation method, and coverage acquisition method.

(3) In addition, the trend of coverage of 128 different genotypes of wheat was showed through data collection. The coverage of wheat with high nitrogen fertilizer at the seedling stage was lower.

(4) Finally, the causes of severe freezing injury and the extraction of mild freezing injury were analyzed. These causes included the phenomenon of lacking seedlings, the withering and yellowing of leaves, and the discrimination of freezing injury degree under mild freezing injury.

In general, the automated phenotypic analysis platform provided a solution for high-throughput phenotypic research of field wheat freezing injury. It quantified the freezing stress and had a certain guiding significance for wheat breeding.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Hongwen Zhang and Jieyu Xian: Conceptualization; Yang Bai and Xiuqing Fu: Methodology; Yang Bai: Writing Original Draft Preparation; Jing Zhou and Hongwen Zhang: Writing—Review and Editing. All authors read and approved the final manuscript.

Funding

Jiangsu Agricultural Science and Technology Innovation Fund (Grant number CX (20) 3085)

Fundamental Research Funds for the Central Universities (Grant number KYXJ202002)

Availability of data and materials

Not applicable

Declarations

Declarations Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicts of interest.

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Figures

Figure 1

Mobile phenotype cabin in field and wheat regionalization

Figure 2

RGB FREEZING INJURY SYSTEM hardware overview. This system is used to obtain phenotypic parameters of different wheat species. Names for A1-D4.

Figure 3

Software interface of the phenotyping system (A) Device software interface. (B) Parameter setting and imaging page.

Figure 4

The image processing pipeline

Figure 5

RGB image segmentation process.

Figure 6

The frozen part is not included in the coverage range (a) the physical image (b) The mark of the withered and yellow part of the leaves due to freezing injury was not identified

Figure 7

Comparison of vegetation coverage of different wheat genotypes under the three nitrogen treatments

Figure 8

Original and segmented maps of different levels of coverage (frost damage level)

Figure 9

Frost damage rating of wheat in the following: (a) physical map of n1, 0 kg/hm2 nitrogen fertilizer treatment and wheat grading of different genotypes; (b) physical map of n2, 180 kg/hm2 nitrogen fertilizer treatment and wheat grading of different genotypes; and (c) physical map of n3, 240 kg/hm2 nitrogen fertilizer treatment and wheat grading of different genotypes.

Figure 10

box diagram of vegetation coverage and visual experience frost damage classification

Figure 11

Display of seedling shortage. (a) Comparison of different degrees of freezing injury in residential areas (b) Freezing damage area display (c) The situation of wheat dead seedlings (d) Seedling shortage rate

Figure 12

There were significant differences in vegetation coverage between representative frost resistant and non-frost resistant genotypes. Different letters on the bar graph indicate significant differences, as determined by LSD ($P \leq 0.05$)

Figure 13

Pseudo color map of wheat in light freezing injury area, as follows: (a) freezing injury area, (b) withered yellow leaf extraction, and (c) actual image of withered yellow leaf extraction