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Dissection of environmental and physiological effects on the temperature difference between superior and inferior spikelets within a rice panicle

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ABSTRACT

Temperature of a plant organ constitutes an integrative index to its eco-physiological properties and status. However, little attempt has been made to dissect the combined effect of ecological and physiological factors on the surface temperature of a plant organ such as the rice spikelet. In this study, using a deactivated plant as reference, we developed a novel comparison method to dissect the environmental and physiological effects on temperature of rice spikelet. Three *japonica* rice cultivars with contrasting canopy features were used as testing materials. Temperatures of flag leaf, superior and inferior spikelets and their diurnal rhythm during grain filling stage were precisely measured by a hand-held infrared thermometer. The results showed that the variation of environmental conditions within a panicle was relatively minor, posing a limited influence on temperature difference between the superior and inferior spikelet. On the other hand, it was the intrinsic physiological properties that considerably affected the spatial variations of spikelet temperature within a panicle. Chemical analysis of sucrose and starch in grains and bracts indicated that the superior spikelet is more physiologically active at photosynthetic assimilation and starch biosynthesis. Interestingly, sugar in bracts exhibited a pattern of diurnal changes similar to the source leaf but different from the sink grain, confirming that bracts are source organs for grain filling. Our findings yield penetrating insight into the eco-physiological foundation of spikelet temperature, thus being helpful for the application of physiological approaches in crop breeding for cooler canopy.

1. Introduction

Current estimates show that nearly 690 million people or 8.9 percent of the world population are under the threat of malnutrition, and if recent trends continue, the number of people affected by hunger would surpass 840 million by 2030 [1]. It is thus urgent to enormously boost crop production to meet the requirement of an ever increasing population, which is projected to be nearly 10 billion at the middle of this century [2]. However, after a quantum leap in production by the Green Revolution during the 1960s, followed by limited progress during the late 1990s, it is apparent that increasing crop productivity is a daunting task to meet the burgeoning demographic demand, especially in the face of climate change [3,4].

Physiological breeding, employing physiological traits (PTs) like canopy temperature (CT), stomatal conductance (Gs), carbon isotope discrimination (CID) as selection criteria, has been successfully applied in wheat breeding programs by CIMMYT and in Australia [5]. It has been recognized as a helpful complement to conventional and molecular breeding, in particular for the areas that suffer drought and heat stresses, the two greatest challenges for adaption of crops to future climate scenarios [6]. Despite a set of PTs as targets for breeders, more traits with sound physiological foundation and clear indicative meaning are needed to improve the efficiency of germplasm screening and early generation selection.

CT is an exemplar of PTs that is generally accepted and incorporated into crop breeding. Physiologically, it reflects the amount of transpiration that has an effect on evaporative cooling, and is cor-

related with many physiological factors including Gs, CID, water use efficiency, leaf area index, root depth, and eventually crop yield [7–9]. In addition, it is an integrative measurement by scoring the entire canopy of a crop stand, at which level crop yield is determined [10]. Lower CT is positively associated with yield even grain quality under heat and drought stress, indicating a better fitness to the changing environments [11]. In rice, Caine et al. [12] engineered the high-yielding cultivar 'IR64' to produce fewer stomata by overexpressing the epidermal patterning factor *OsEPF1*. The resulting plants had lower CT under drought and was more conservative in water use. CT has been used as a parental selection tool for strategic crossing and in early generation selection, culminating in consistent genetic gains in heat and drought stressed environments, as well as raising yield potential by CIMMYT and collaborators [5]. Although widely applied, CT has some drawbacks to be addressed [6]. First, the measurement of CT is a mixture of organs of source (leaf) and sinks such as wheat spike or rice panicle, which have contrasting physiological activities (respiration, transpiration, etc.) and organ temperature. In addition, CT is sensibly affected by environmental variables including incident radiation, wind, and humidity, which in combination mask the effect of physiological processes. Therefore, it is necessary to dissect the effects of environmental conditions and physiological activities, in order to fully elucidate the mechanism underlying the useful index of PT in crop breeding.

In architecture, the canopy of rice stand is quite different from that of wheat, regarding the relative position of leaf to panicle/spike. Generally, spikes of wheat stretch above the canopy. By contrast, panicles of indica rice are usually covered by leaves, while those of *japonica* rice are above, under or at a similar height with flag leaf, depending on genotypes [13]. Morphologically, rice panicle and wheat spike are quite different, in particular the palea, lemma, and glumes of rice are closed after anthesis, whereas those of wheat are open, with the grain exposed to the air. Collectively, the variations in canopy and organ architecture indicate different mechanisms controlling the CT of rice and wheat, which still remains elusive.

So far, research on the temperature of the rice panicle has been mainly on its relation to spikelet fertility under heat stress. Results showed the combination of high air temperature and high humidity had a marked influence of increasing panicle temperature, resulting in high occurrence of spikelet sterility and lower grain yield [14–16]. Temperature of a plant organ is indicative of its eco-physiological properties and status. However, little attempt has been made to dissect the compound effect of ecological and physiological factors on the surface temperatures of a plant organ such as the rice spikelet. More work is required especially the three fundamental issues as follows: (i) the dissection of environmental and physiological effects on CT to further understand factors controlling CT and its relation to grain yield; (ii) the spatial heterogeneity of temperature distribution within rice canopy, between leaf and panicle as well as between the superior and inferior spikelet within a panicle; and (iii) the diurnal changes of CT and its relevance to the disproportional increase in night temperature, a feature of global warming [17]. In addition, plants have a regulatory mechanism of temporarily transforming sucrose into starch to cope with daily change of light [18]. However, it remains open whether there exits a circadian rhythm in sucrose and starch levels for the bracts of rice spikelet, and the physiological meaning of this changing pattern is still unclear.

In this study, using a deactivated (dead) plant as reference, we developed a novel comparison method for dissecting the environmental and physiological effects on rice panicle temperature. Three *japonica* rice cultivars with contrasting canopy features were selected as testing materials. Temperatures of flag leaf, superior and inferior spikelets and their diurnal rhythm during grain filling

stage were precisely measured by a hand-held infrared thermometer (IRT). Our aims are to (i) evaluate the effect of physiological activities inside rice spikelet on its apparent temperature; (ii) explore the temperature difference between superior and inferior spikelet; and (iii) detect the diurnal change of external temperature of rice spikelets and relate it to the internal physiological activities, starch and sucrose levels. Collectively, we hope our findings should afford fresh insight into the mechanism underlying CT, thus broadening the application of CT in breeding programmes under the scenario of climate changes.

2. Materials and methods

2.1. Materials and planting

The experiments were conducted at Danyang experimental station (31°54′31″N, 119°28′21″E) in 2018 and 2019. Three *japonica* rice cultivars with contrasting canopy architecture were selected according to the leaf to panicle ratio (LPR) in terms of light interception calculated by software developed by the authors (Fig. 1) [19]. Among them, Wuyujing 3 (WYJ3) has the lowest LPR (0.95) at 15 DAA (days after anthesis). It is a panicle-dominating genotype, with panicle being above the leaves. Wuyunjing 7 (WYJ7) has the highest LPR (3.31) and is leaf-dominating with panicle under the leaves. Ningjing 8 (NJ8) is the intermediate type, with a LPR of 2.79. Of note is that the plant architecture varies with phenological stages, showing a transformation of compact and erect type into loose and curved one, which is associated with the bending by filled grains (Fig. 1).

The soil type of the pot experiment was clay loam with pH 6.41, 16.37 g kg^{-1} organic matter, 0.93 g kg^{-1} total N, 0.54 g kg^{-1} total P. Plastic pots, 30 cm in height and 34 cm in diameter, were filled with fine-grained soil, about 20 kg per pot in 2018 and 15 kg per pot in 2019. The plants were fertilized twice during growth period. Before transplanting, 1.0 g N, 0.5 g P_2O_5, and 0.8 g K_2O were applied to each pot as basal fertilizer, and the same fertilization rate as top-dressing at panicle initiation stage. Seeds were sown in the nursery bed on May 21 in 2018, and on May 26 in 2019. After 4 weeks, seedlings were transplanted into the pots. Each pot had seven hills with two seedlings per hill. Plants with similar flowering date were selected and tagged for measurement and sampling.

2.2. Control experiment to dissect environmental and physiological effects

Three days before temperature measurement, plants with typical height and flowering day were chosen as reference plants. The shoot was cut at the bottom internode to deactivate the metabolic activity of the plant. The resulting shoot was inserted back to its original position (Fig. 2). As shown in Fig. 3, we assume that the temperature of an active spikelet (T_s) is the combination of environmental (T_e) and physiological effects (T_p). By contrast, for the reference plant without physiological activities, the measured spikelet temperature is merely the T_e (Fig. 2). Therefore, it is feasible to dissect T_e and T_p by this control experiment.

2.3. Temperature measurements

Temperature of leaf, superior and inferior spikelets was measured by an infrared thermometer with a target area of less than 1 mm^2 (Optris LS, Optris, Germany). Superior spikelet refers to those on the primary branches of the upper and middle rachis, while inferior spikelet on the secondary branches of the bottom and middle rachis within a panicle, as illustrated in our previous study [20]. Starting on 5 DAA, the measurement had an interval
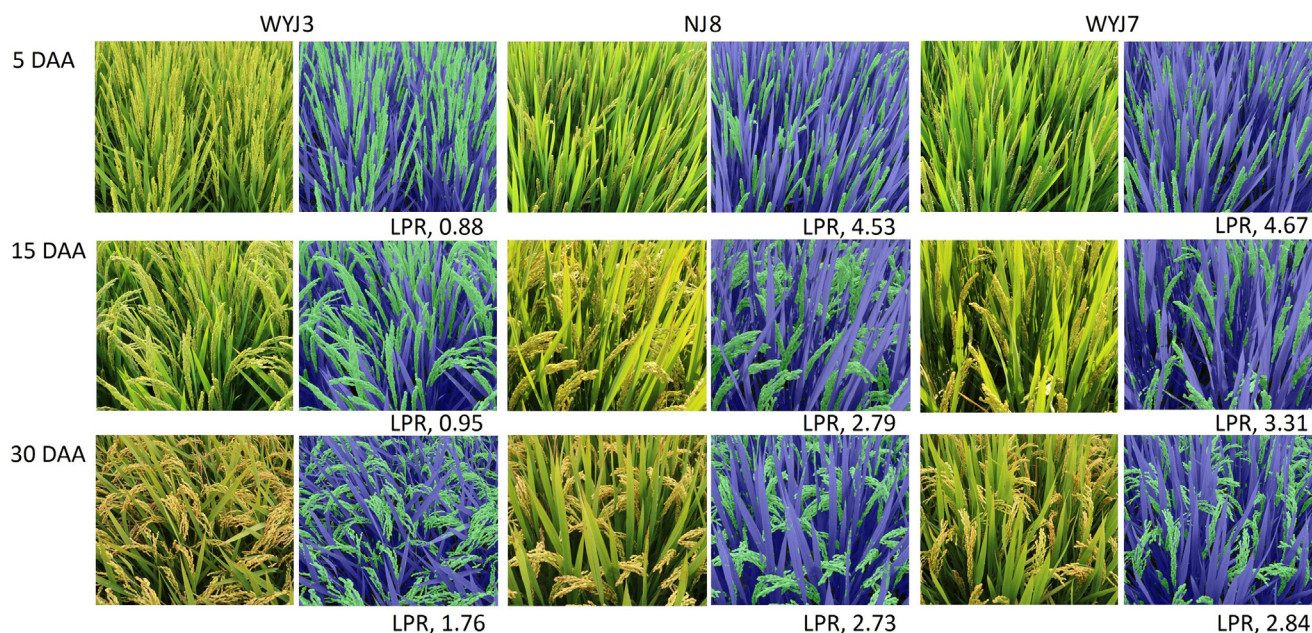
Fig. 1. Original and segmentation (blue and green) images of the three *japonica* cultivars selected at three phenological stages. For each cultivar, a representative photo is shown. LPR value is the mean calculated from three photos of the rice crop stand. DAA, days after anthesis; LPR, leaf to panicle ratio in terms of light interception.

Fig. 2. The tested active plants and the reference plants. White arrows refer to the deactivated reference plant. NJ8 was used for representation. T_s, temperature of an active spikelet; T_e, temperature associated with environmental conditions; T_p, temperature associated with physiological activities.

of five days in 2018 and ten days in 2019. Measurements were conducted at sunrise, 9:00, 12:00, 15:00, sunset, and 24:00 of a day, with sunrise and sunset time varying with phenological stages at the experimental site. Air temperature was measured by an automatic recorder (HOBO Pro v2, Onset, USA), installed near the pot experimental site, recording the air temperature at a height of 1.5 m every 5 min. Meteorological conditions of temperature measuring dates including air temperature, solar irradiation, and relative humidity are presented in Tables S1 and S2, and Fig. S1.

2.4. SEM observation of stomata of leaf and spikelets

Flag leaf, superior and inferior spikelets were sampled at 10 DAA. The samples were cut into the small pieces with a razor blade to produce a fracture rather than a clear cut, with a piece size of less than 6 mm². Cleaned with phosphoric acid buffer solution and dehydrated by ethanol, the samples were put into isoamyl acetate solution and dried at the critical point of CO_2. The dried sample was adhered to the copper sample table with double-sided tape paper. The fracture was sputter-coated with gold in
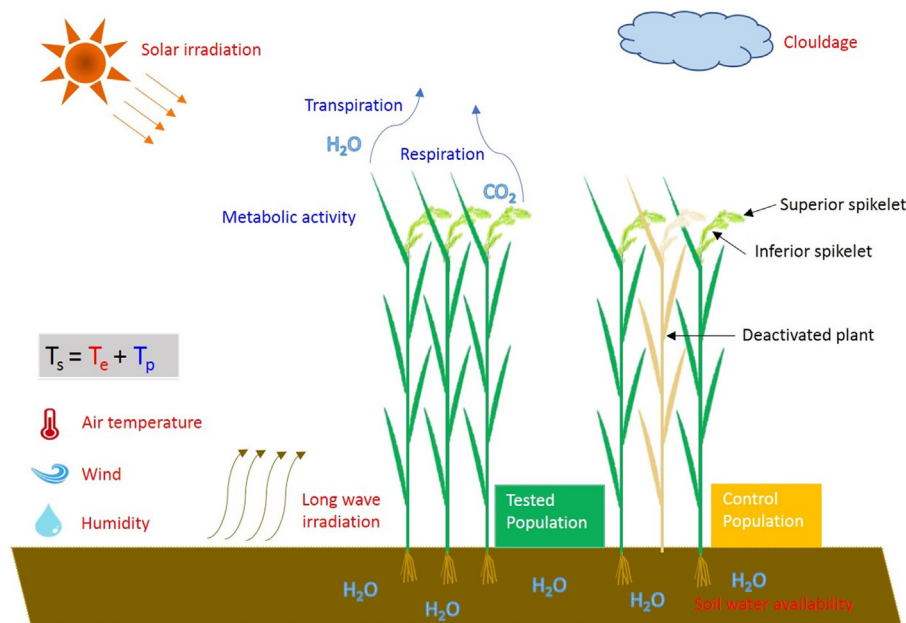
Y. Li, Q. Zhou, M. He et al.

Fig. 3. Diagram of the dissection of environmental and physiological factors affecting the temperature within a rice panicle. Environmental factors are shown in red, including solar irradiation, air temperature, wind, relative humidity, and long wave irradiation from the soil. Physiological factors, blue color, consist of transpiration, respiration, and metabolic activities like starch biosynthesis in the grains.

vacuum and observed by a scanning electron microscope (SEM; S-3000N, Hitachi, Japan) at an accelerating voltage of 15 kV. Stomata on the surface of flag leaves and on the internal surface of lemmas were photographed. For measurement of stomata density and size, 20 representative photos were selected, and number of the stoma and their length and width was examined and measured.

2.5. Starch and sucrose analysis

The panicle and flag leaf of representative plants were sampled at early, middle, and late stages of grain filling, being September 5th to 7th, 16th to 18th, and 24th to 26th, respectively, in 2019. Meteorological conditions of sampling dates are presented in Fig. S1. In general, the sampling was conducted on sunny days, meaning active photoassimlate production occurred in the photosynthetic organs of leaves and bracts. The samples were ground into powder and stored in the freezer at −80 °C until analysis.

For sucrose analysis, approximately 0.1 g sample was extracted with 8 mL 80% aqueous ethanol at 80 °C for 30 min. After cooling, the sample was centrifuged at 5000 r min^{-1} for 15 min and the supernatant was collected in a 50 mL volumetric flask. The extraction process was repeated three times and the supernatants were combined in the flask with addition of distilled water to 50 mL. Decolorized with activated carbon and purified by filtering, 100 μL of 2 mol L^{-1} NaOH solution was added to 1.0 mL of filtered solution, and then the mixture was boiled at 100 °C for 30 min. After cooling, 3.0 mL of 10 mmol L^{-1} HCl and 1.0 mL of 0.1% resorcinol were added. The reaction mixture was incubated at 80 °C for 10 min, and absorbance at 480 nm was recorded. Compared with a standard curve of sucrose, sucrose concentration was calculated as mg g^{-1} fresh weight [21].

For starch determination, two replicates of about 100 mg powder of the same sample were added to the two tubes with 10 mL of 100 mmol L^{-1} sodium acetate buffer, with one as a sample blank and the other the starch testing tube. The testing tube was mixed with 0.1 mL of undiluted thermostable α-amylase, and that of the blank tube 0.1 mL of 100 mmol L^{-1} sodium acetate buffer. Immersed in a boiling water bath for 15 min and water bath at

50 °C for 5 min, 0.1 mL of undiluted amyloglucosidase was added to the testing tube, and 0.1 mL of 100 mmol L^{-1} sodium acetate buffer to the blank. Incubated at 50 °C for 30 min and cooled at room temperature, 2.0 mL of each solution was centrifuged at 13,000 r min^{-1} for 5 min. A 0.1 mL aliquot was mixed with 3.0 mL of Glucose Determination Reagent (Megazyme, Bray, Ireland). Incubated at 50 °C for 20 min, absorbance against the reagent blank was measured at 510 nm. The starch content was calculated as mg g^{-1} fresh weight [22].

2.6. Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using the general linear model. Samples were analyzed in triplicate and mean values were used for comparison. The analysis of variance was performed using Shortest Significant Ranges (SSR) test in software name (Statistical Product and Service Solutions, IBM).

3. Results

3.1. Diurnal variations in temperatures of flag leaf, superior and inferior spikelets

In this study, organ temperature is expressed as temperature difference (TD) between it and its ambient environment, the air temperature detected at 1.5 m height. It should be noted that the results of 2018 and 2019 were similar regarding the TD variations between organs. To save space, the data of 2019 are presented in the main text, while those of 2018 are provided as supplementary materials.

A clear pattern of diurnal changes in organ temperature was revealed, when examining the data of three cultivars across different grain filling stages in 2018 (Fig. S2) and 2019 (Fig. 4). TDs of flag leaf and spikelets increased at sunrise, peaked between 9 AM and 12 AM, and then decreased to the lowest value at sunset. Afterwards, a slight elevation was detected until midnight.
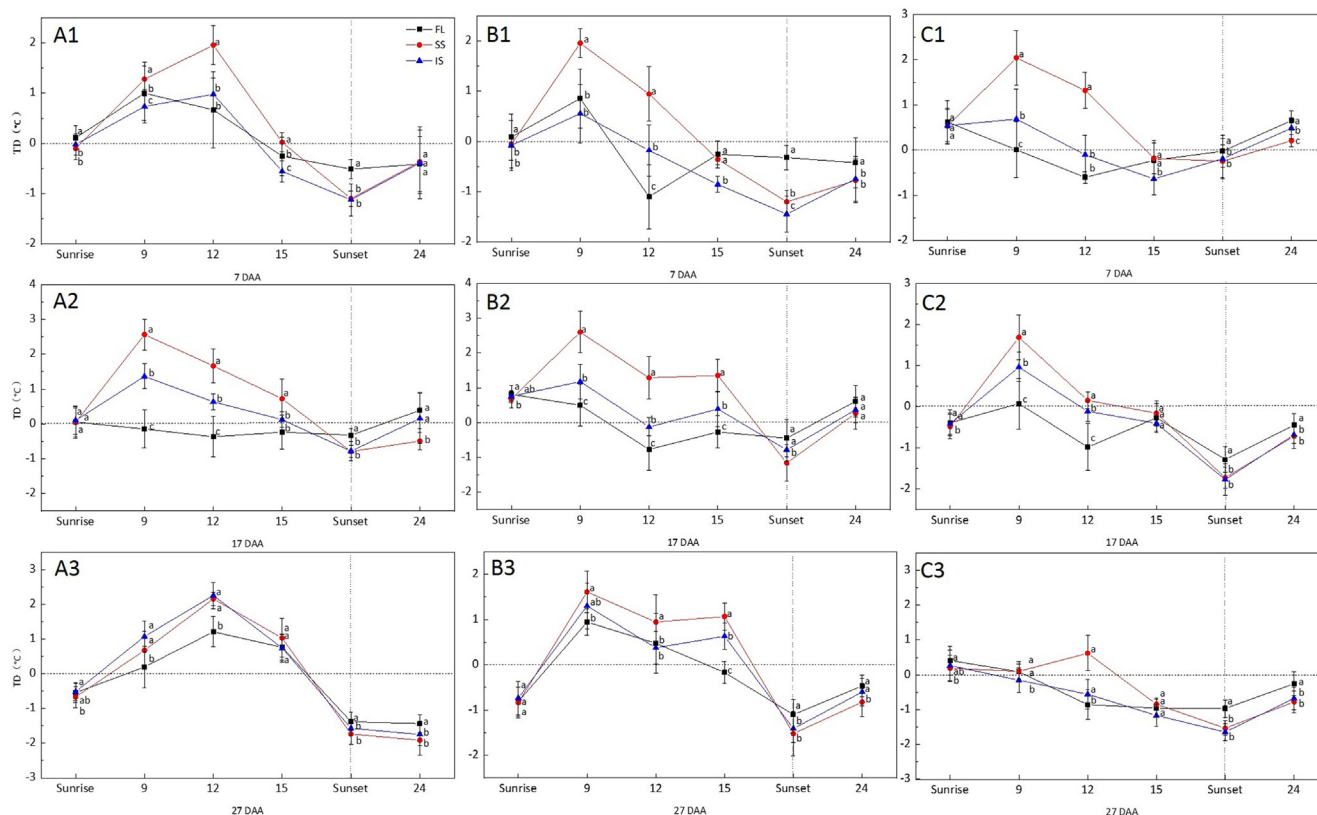
Y. Li, Q. Zhou, M. He et al.

Fig. 4. Diurnal changes of temperature of flag leaf and superior and inferior spikelets during the grain filling stage in 2019. A, B, and C, the cultivars of WYJ3, NJ8, and WYJ7. Data shown are temperature differences (TDs) between plant organ and the air. DAA, days after anthesis. Data of the same date followed by a different letter are significantly different ($P < 0.05$). The same as blow.

Organs differed in surface temperature, with flag leaf being consistently cooler than spikelets at daytime (Fig. 4; Fig. S2). This is partly associated with the powerful effect of transpirational cooling on the leaf, as is evidenced by high density and larger size of stomata on the leaf surface (Fig. 5; Fig. S3). On the other hand, leaf temperature was usually higher than that of the panicle at night (Fig. 4; Fig. S2). This might be associated with the closure of stomata, but the mechanism is still elusive. Generally, the superior spikelet was relatively hotter than the inferior at daytime (Fig. 4). Morphologically, as shown in Fig. S3, stomata density, length and width were similar between the superior and inferior spikelet, suggesting that stomatal anatomy may not be the only main reason causing the TD between spikelets.

TD between the tested organs and the air showed an obvious diurnal rhythm (Fig. 4). Generally, the largest difference occurred on 9 AM or 12 AM for the positive value and at sunset for the negative value, depending on cultivars and phenological stages (Fig. 4). Phenological stage exercised a remarkable influence, with the early and middle stages of grain filling showing the larger variations among organs and larger TD (Fig. 4; Fig. S2). Taking WYJ7 as an example, at 9 AM TDs of flag leaf, superior and inferior spikelets were similar, being around zero, at late stage (Fig. 4C3); but they were markedly different at early and middle stages, with superior spikelet being nearly 2 °C higher than flag leaf (Fig. 4C1, C2). However, genotypic variations existed, with the leaf-dominating type cultivar, WYJ7, showing smaller variations in organ temperature (Fig. 4; Fig. S2).

3.2. Diurnal variations in temperatures of the reference plant

The control experiment with a deactivated plant was performed to estimate the contribution of environmental variables to spikelet temperature. Generally, diurnal changes of temperature of the deactivated spikelet showed a similar trend to those alive, increasing at sunrise, peaking at 9 AM or 12 AM, then reducing until sunset, and finally increasing slightly at midnight (Fig. 6; Fig. S4). Interestingly, at early stage, the position of superior spikelet on the peripheral parts of the upper and middle rachis was warmer than that of the inferior spikelet. On the other hand, the opposite trend existed at middle and late stages. This may be explained by the changing panicle architecture that was erect at early stage but droopy at middle and late stages because of the filled grains, as shown in Fig. 1.

Overall, compared with data of the active plant (Fig. 4; Fig. S2), the reference plant basically had a relatively smaller variation in surface temperature between superior and inferior spikelet, indicating that spikelet temperature of the active plant is more affected by its intrinsic physiological properties. In addition, it suggests the feasibility of the control experiment in dissection of the environmental and physiological effects on the TD between organs.

3.3. Estimation of physiological effect on spikelet temperature

In this study, we presume that the temperature of an active spikelet (T_s) is the sum of environmental effect (T_e) and physiological effect (T_p), as illustrated in Fig. 3. Therefore, we can estimate the physiological effect by subtracting the environmental effect quantified by the control experiment (Fig. 6; Fig. S4). It is obvious that the estimated T_p of superior spikelet is consistently higher than that of the inferior, with fewer exceptions as shown in Fig. 7A3, and Fig. S5A4 and C2. In addition, the temperature difference in T_p persisted along the grain filling process, not just at the early and middle stages like T_s and T_e.
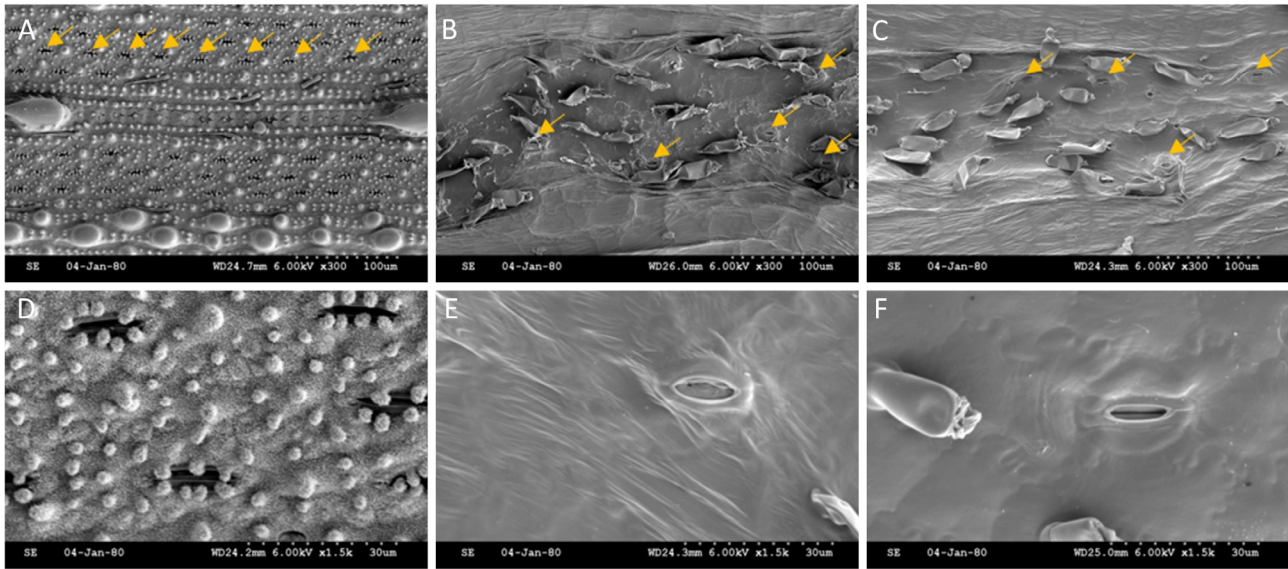
Fig. 5. Scanning electron micrograph of surface structure of flag leaf (A, D) and the lemma of superior spikelet (B, E) and inferior spikelet (C, F). Yellow arrows refer to stomata. The bottom row is the magnification of the upper, showing the morphological features of stomata.
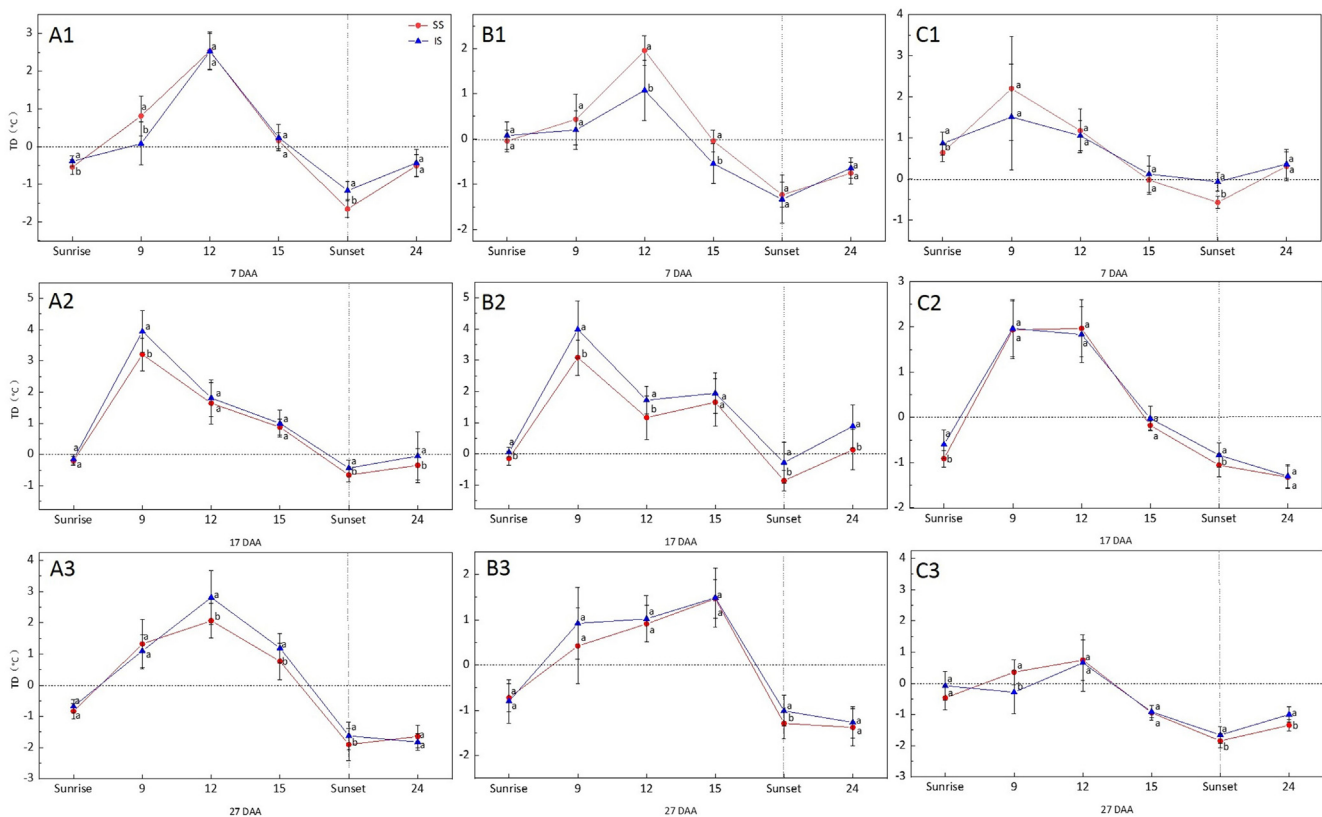

Fig. 6. Diurnal changes of temperature of the reference plant during grain filling stage in 2019. A, B, and C, the cultivars of WYJ3, NJ8, and WYJ7. Measurements were conducted at the positions of superior spikelet (SS) and inferior spikelet (IS) that had lost physiological activities. Data are temperature differences (TDs) between the deactivated organ and the air, an estimation of T_e.

3.4. Diurnal changes of starch and sucrose in flag leaf, superior and inferior grains, and their bracts

The pattern of diurnal changes for organ temperature is a reflection of its physiological activity. We investigated the starch and sucrose contents in flag leaf, superior and inferior grains, and their bracts, and found a circadian cycle of sucrose in flag leaf and bracts of both superior and inferior spikelets (Fig. 8A and C). Sucrose in the flag leaf increased at sunrise and peaked at sunset. Afterwards it decreased until sunrise the next morning. Similarly, the bracts had higher content of sucrose at daytime than at night. By contrast, no obvious circadian rhythm was observed with the grains
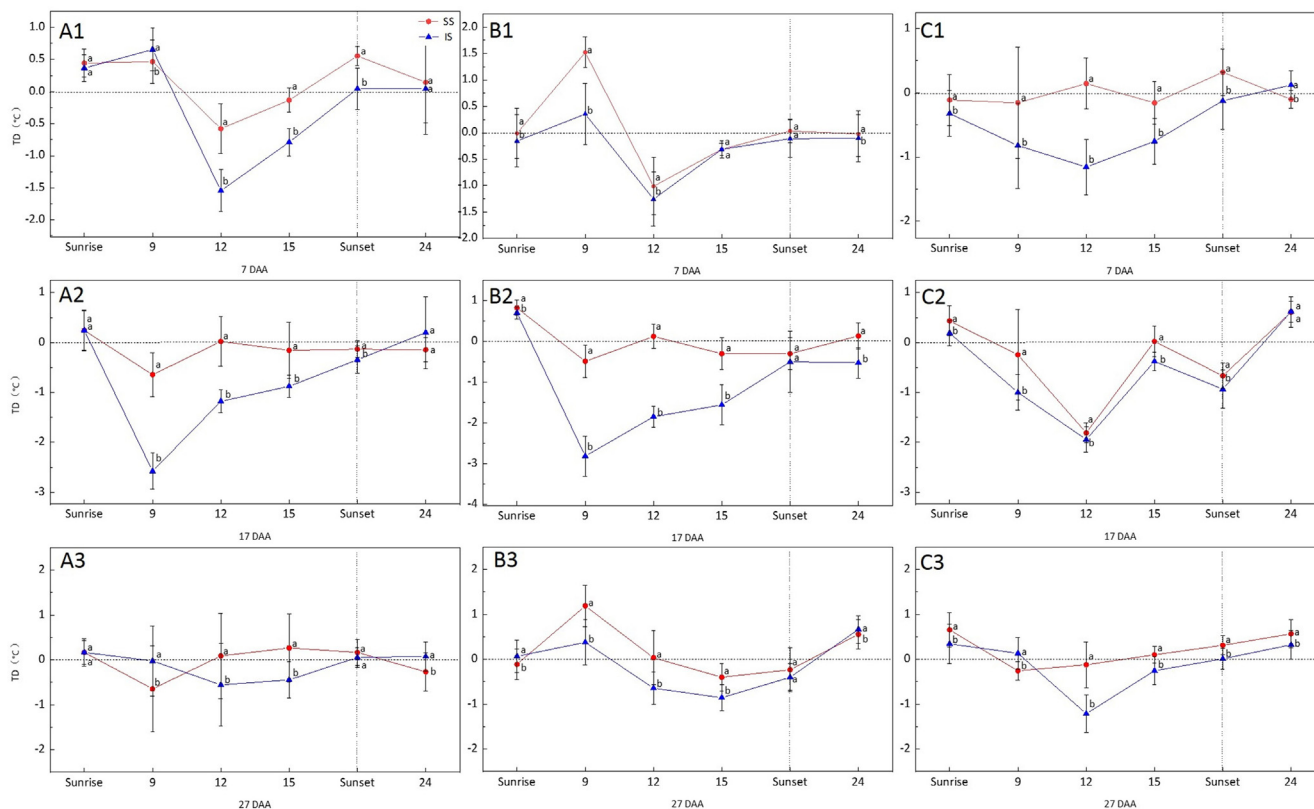
Fig. 7. Estimation of physiological effect on spikelet temperature during the grain filling stage in 2019. A, B, and C, the cultivars of WYJ3, NJ8, and WYJ7. As represented in Fig. 3, the temperature of an active spikelet (T_s) is the sum of environmental effect (T_e) and physiological effect (T_p). Data in the figure are T_p, calculated by subtracting temperature in Fig. 6 (T_e) from its counterpart in Fig. 4 (T_s).

(Fig. 8B). Therefore, the bracts are more like a source organ (leaf) than a sink organ (grain) in terms of photoassimilate production.

At early stage of 10 DAA, sucrose content tended to be lower in inferior grain than superior grain at sunrise, while it was higher at late stage of 30 DAA (Fig. 8B). By contrast, at the middle stage of grain filling (20 DAA), inferior grain contains higher sucrose content than the superior, as also reported by a previous study, [23] supporting the hypothesis that sink limitation is one of the main causes of poor grain filling of inferior spikelet. Bracts of superior spikelet were higher in sucrose content than those of the inferior at 10 and 20 DAA, suggesting a higher photosynthetic activity in the superior spikelet. However, at 30 DAA, sucrose content was lower in the superior spikelet due to more senescence.

The transient storage of starch in leaves showed a circadian cycle, but not in the bracts and grains (Fig. 8D–F). Superior grain had a larger amount of storage starch than the inferior, indicating the strong activities of starch biosynthesis in it.

4. Discussion

4.1. Comparison of environmental and physiological effects

CT is a function of external conditions around the crop stand and intrinsic or physiological properties inside the crop plant [6]. Environmental conditions such as light interception, wind speed, and relative humidity have strong influence on CT, and therefore the measurement of CT requires relatively cloud-free, windless days to obtain reliable data. The plant itself also significantly affects CT. Physiological properties like vascular system, stomatal aperture, metabolism, and source and sink balance collectively contribute to transpirational cooling. Further, CT is subject to

genetic control by multiple genes, associated with root development [24] and spikelet fertility [25]. Therefore, CT is a diagnostic index of the ability of crop adaptation to environmental stress. However, the relative contribution of environmental and physiological factors to CT has not been quantitatively assessed so far. It is necessary to dissect the effect of the two factors in order to acquire a definite meaning of the apparent temperature that is measured by infrared thermometer. In this study, the use of deactivated plants makes it possible to compare the contribution of environments and physiological properties to TD between spikelets (Fig. 6; Fig S4). Our results showed that the fluctuation of environmental conditions within a panicle was relatively minor, posing a limited influence on the difference in surface temperature of spikelets on upper and lower rachis. In other words, it was the intrinsic physiological properties that essentially determine the spatial variations of spikelet temperature within a panicle. As far as we know, this is the first attempt to dissect the environmental and physiological effect on plant organ temperature. Our findings highlight the physiological significance of panicle temperature, and has implications in eco-physiological studies and crop breeding, as discussed below.

Nevertheless, there exists limitation in the method proposed by us, because of the daunting complexity of the interaction between environmental conditions and physiological activities within a crop stand. For example, in the reference plant, we observed that a slight increase in panicle temperature occurred at night. This might be attributed to the longwave irradiation from the soil under the canopy. Supporting this, the position of inferior spikelet that is proximate to the ground compared with the superior, tended to be warmer at night, even for the early stage when it was cooler at daytime (Fig. 6A1, B1 and C1; Fig. S4C1). However, the panicle temperature is not solely related to the longwave irradiation from the soil,
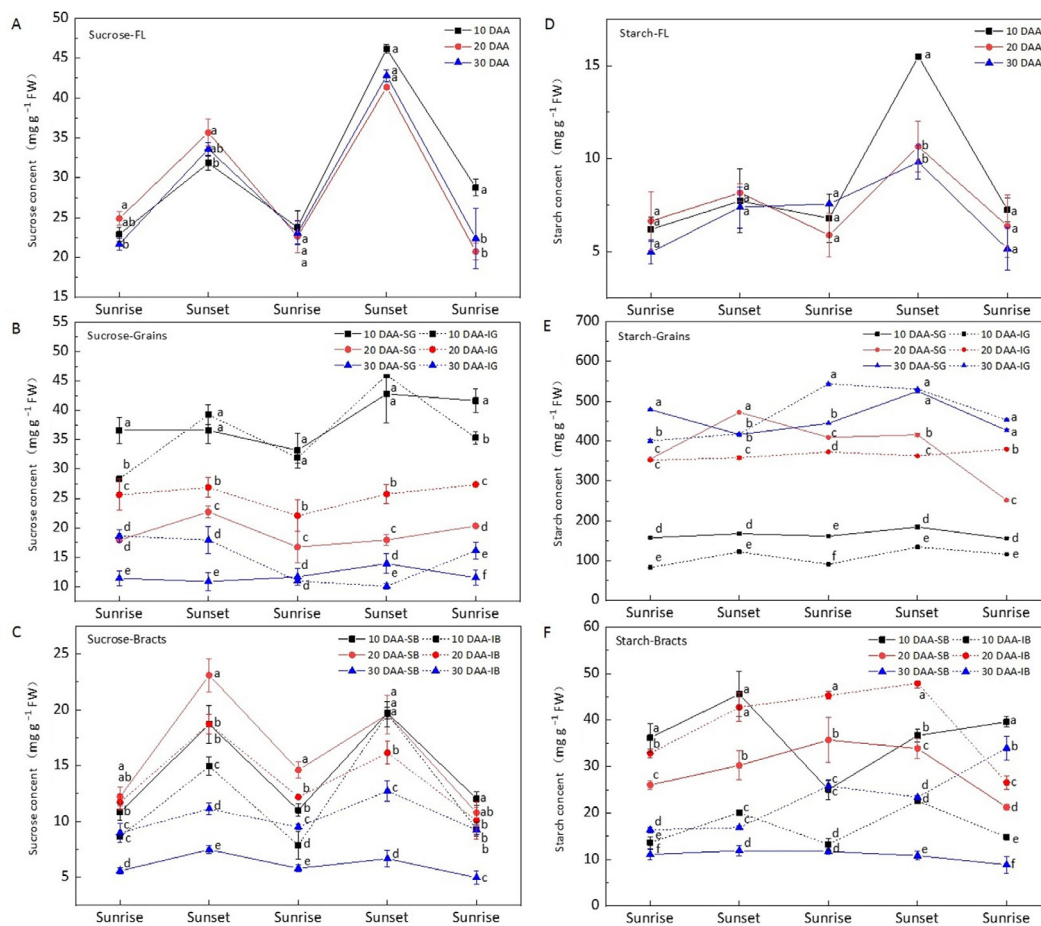
Fig. 8. Diurnal changes of starch and sucrose in flag leaf (FL), superior grain (SG), and inferior grain (IG), and their bracts during grain filling stage in 2019. IB, bracts of inferior spikelet; SB, bracts of superior spikelet.

in that case the panicle temperature should be higher than the air temperature. On the contrary, spikelet temperature of the reference plant was lower than the air temperature at night (Fig. 6), suggesting that panicles of the controlled plants are cooled down by the adjacent active plants. To testify this hypothesis, a reference canopy rather than a reference plant should be created and used in future studies.

4.2. Physiological significance of spikelet temperature

Within rice panicles, superior spikelets flower earlier, fill faster and produce larger and heavier grains compared to inferior spikelets. Modern rice cultivars with large panicles usually have a serious obstacle of poor grain filling of later-flowering inferior spikelets, associated with source activity, [23] sink strength [20,26] and the capacity of assimilate transport from source to sink [27]. In addition, hormones including ABA and ethylene, and transcriptional factors like AP2 and NAC have been reported to actively participate in the regulation of the grain filling process of inferior spikelets [28]. Nevertheless, the mechanism underlying the incomplete filling of the inferior spikelet is not well understood. In this study, we provide a new perspective of organ temperature to address this problem. By subtracting the effect of environmental conditions, the physiological effect on surface temperature of superior and inferior spikelet was uncovered, with the temperature 'generated' by physiological activities of inferior spikelet being significantly lower than that by superior spikelet at day time. Although morphological observation showed that stomata density

and size were similar between the superior and inferior spikelet, it not excluded the possibility of the involvement of transpirational cooling in regulation of TD between spikelets. Physical properties of stomata is only one of the factors contributing to transpiration cooling, other factors like the stomatal resistance, the boundary layer resistance, and the difference in water vapor inside and outside the lemma cell also control the intensity of transpiration. Future studies should be centered on this process of spikelet transpiration. Our results also showed that bracts of inferior spikelet were lower in sucrose content than those of the superior at early and middle stages of grain filling, suggesting the weaker photosynthetic activity in the inferior spikelet. In addition, inferior grain had more starch but less sucrose, implying weaker activities of starch biosynthesis in inferior grain. It is thus inferred that the inferior grain are less physiologically active at photosynthetic assimilation and anabolism of starch. On the other hand, the difference is not significant at night, indicating a similar metabolic activity between them when photosynthesis ceases. These findings confirm that low sink strength is a major contributor to poor grain filling of inferior spikelet.

During a 24-h day and night cycle, there are large fluctuations in light intensity and temperature, requiring plants to coordinate biological processes with environmental light–dark and temperature cycles. Plants have evolved a regulatory mechanism of partitioning of photoassimilates between sucrose and starch to predict and cope with daily and seasonal changes [18]. In the daytime, CO_2 fixed by the leaf, in the form of soluble sugars, is partitioned between sucrose for export and transient starch for

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Y. Li, Q. Zhou, M. He et al.   The Crop Journal xxx (xxxx) xxx

storage; at night, starch is consumed to provide a carbon source for plant growth [29]. In this study, sucrose in the leaf was higher during the day and lower during the night. Interestingly, bracts exhibited a pattern of diurnal changes similar to the source organ leaf but contrasting to the grains, although they are more closely interlinked by vascular tissue. This finding provides new evidence supporting bracts as source organs for grain filling. For the temporal variations of temperature, both superior and inferior spikelets tended to be warmer during daytime but cooler at night than their surrounding environments, and the differences between them was not significant at night. It was projected that disproportional increase in night temperatures would reduce the diurnal amplitude, causing a series of negative impacts on crop production [30]. In this scenario, the effect of high night temperature on spikelet temperature and grain filling needs to be thoroughly investigated.

4.3. Application of panicle temperature in crop breeding

CT was first reported for detecting genotypic variations in wheat by Blum and co-workers in 1982, and subsequently accepted as an excellent predictor of yield in hot, irrigated environments [31]. Traditionally, CT is measured by hand-held infrared thermometer, with advantages of being non-destructive, non-invasive, and high throughput [32]. The availability of thermal cameras triggered a boom in methods and applications of thermal remote sensing (thermography) in CT measurement in the 2000s [33,34]. Recently, a new airborne thermal imaging platform was established to measure CT in a large-scale field [35]. Overall, these methods integrate a large area of crop population, consisting of spike, peduncle, and leaf, and are more suitable for wheat. However, it may be not applicable for rice, because of the differences in canopy architecture between rice and wheat, as discussed in the introduction section. In this study, marked differences in temperature were detected between leaf and panicle, with the leaf being cooler at daytime whereas warmer at night than the panicle. Thus the IRT-measured CT of a rice population is a mixture of leaf and panicle, the effect of individual organ is masked.

To precisely clarify the adaptive mechanism of rice plant to heat stress, separately measuring temperatures of the leaf and panicle is indispensable. Genotypic differences in panicle temperature were revealed by Yan et al. [16], showing that cultivars with erect panicles are cooler than those with droopy panicles, and *japonica* rice panicles were cooler than those of indica. Similarly, genotypic differences were detected among the three *japonica* cultivars with contrasting canopy architecture, showing a strong interaction with phenological stages (Tables S3–S6). Therefore, a large population of diverse genotypes including landraces, modern cultivars, and advanced breeding lines is needed to screen for elite germplasm for rice breeding. In addition, when applying this trait, care should be taken concerning the timing within a measuring day and phenological stages. According to our study, it is suggested that it should be measured between 9 am and 12 am before 25 DAA, when the difference among organs is more noticeable and measurable, as shown in Fig. 4 and Fig. S2.

5. Conclusions

Using a deactivated plant as reference, this study dissected the compound effect of environmental and physiological factors on TD between rice spikelets. The results showed that the position within the panicle had a relatively smaller influence on TD between the superior and inferior spikelet, while the physiological activities had a larger effect. Chemical analysis of sucrose and starch indicated that the superior spikelet is more physiologically active at

photosynthetic assimilation and starch biosynthesis. Interestingly, sugar in bracts exhibited a pattern of diurnal changes similar to the source organ leaf but contrasting to the grains, suggesting that they are source organs for grain filling. Our findings highlight the physiological significance of spikelet temperature, and widens our knowledge of the eco-physiological mechanism underlying CT, thus being beneficial to the application of CT in crop breeding.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data for this article can be found online at https://doi.org/10.1016/j.cj.2020.10.016.

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