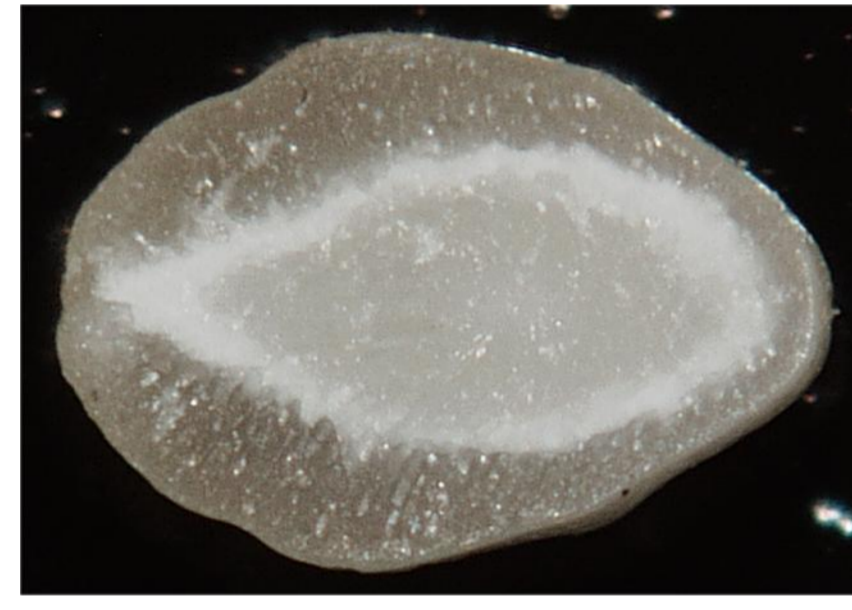


Introduction

Grain filling stage is one of the most sensitive stages to many environmental stresses, such as high temperature, insufficient light, and water deficit. These stresses often cause disruptions of yield and quality of crops. In rice (*Oryza sativa* L.), grain chalkiness is a typical physiological disorder, which exhibits loosely packing of starch granules in the endosperm cells in the kernels and creates air spaces between starch granules to cause a random reflection. It is known that chalkiness is caused by not only high temperature or insufficient light, but also typhoon/foehn like high-speed wind.

In 2007, the early cultivar, Koshihikari plants grown in South Kyushu, Japan were imposed to approximately one day of foehn after low light intensities in ripening stage, and a substantial increase of chalky kernels was observed at harvest. In the chalky kernels, ring-shaped chalky kernel, known as milky-white rice (MWR) kernel (see below), were found to be predominant, reaching up to 45% of total ripened kernels, resulting in disruption of rice appearance. However, little was known about the physiological mechanisms of chalky formation in the kernels under the dry wind condition, aside from the shade effect.

In this work, we conducted several experiments in both field and growth chamber to study the physiological cause of the ring-shaped chalkiness increased by dry wind condition by combining pressure chamber measurements with *in situ* P assay in the endosperms. We discussed the effect of dry wind on kernel development and chalky formation from the viewpoint of water relations.

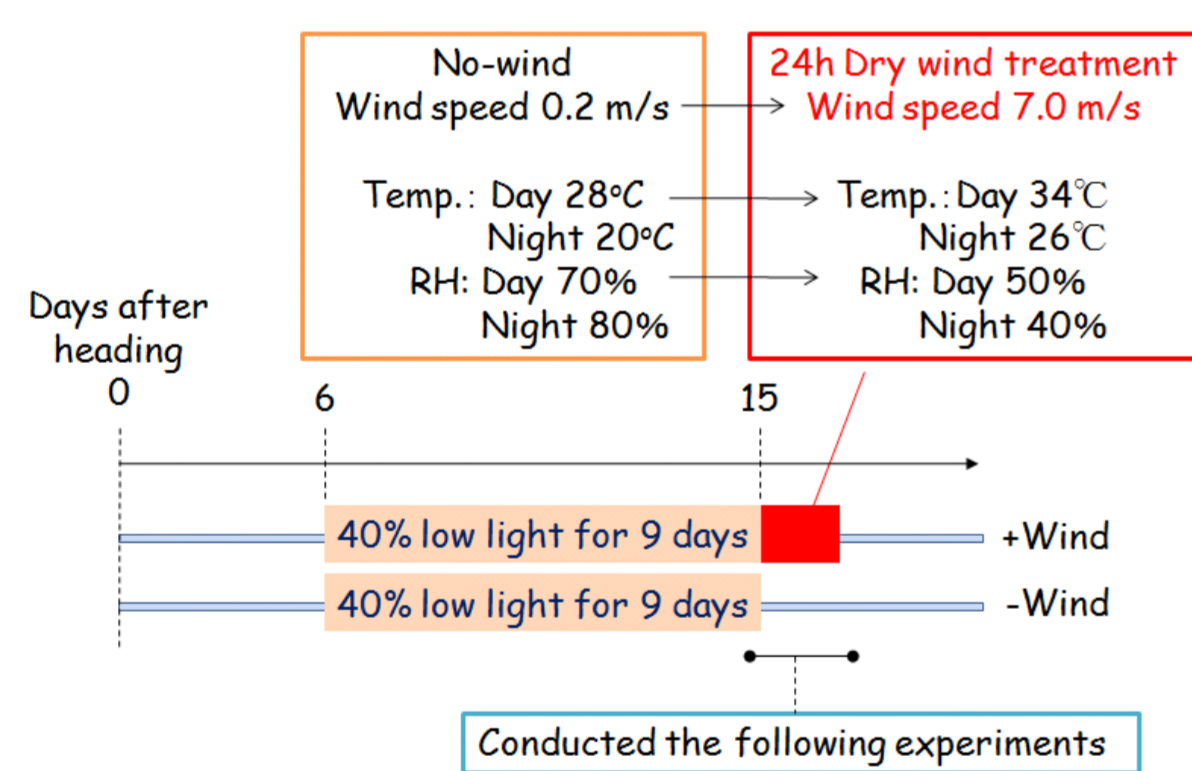


Transverse section of the typical milky white rice kernel produced in 2007, which exhibited a ring-shaped chalkiness in the endosperm.

Methods

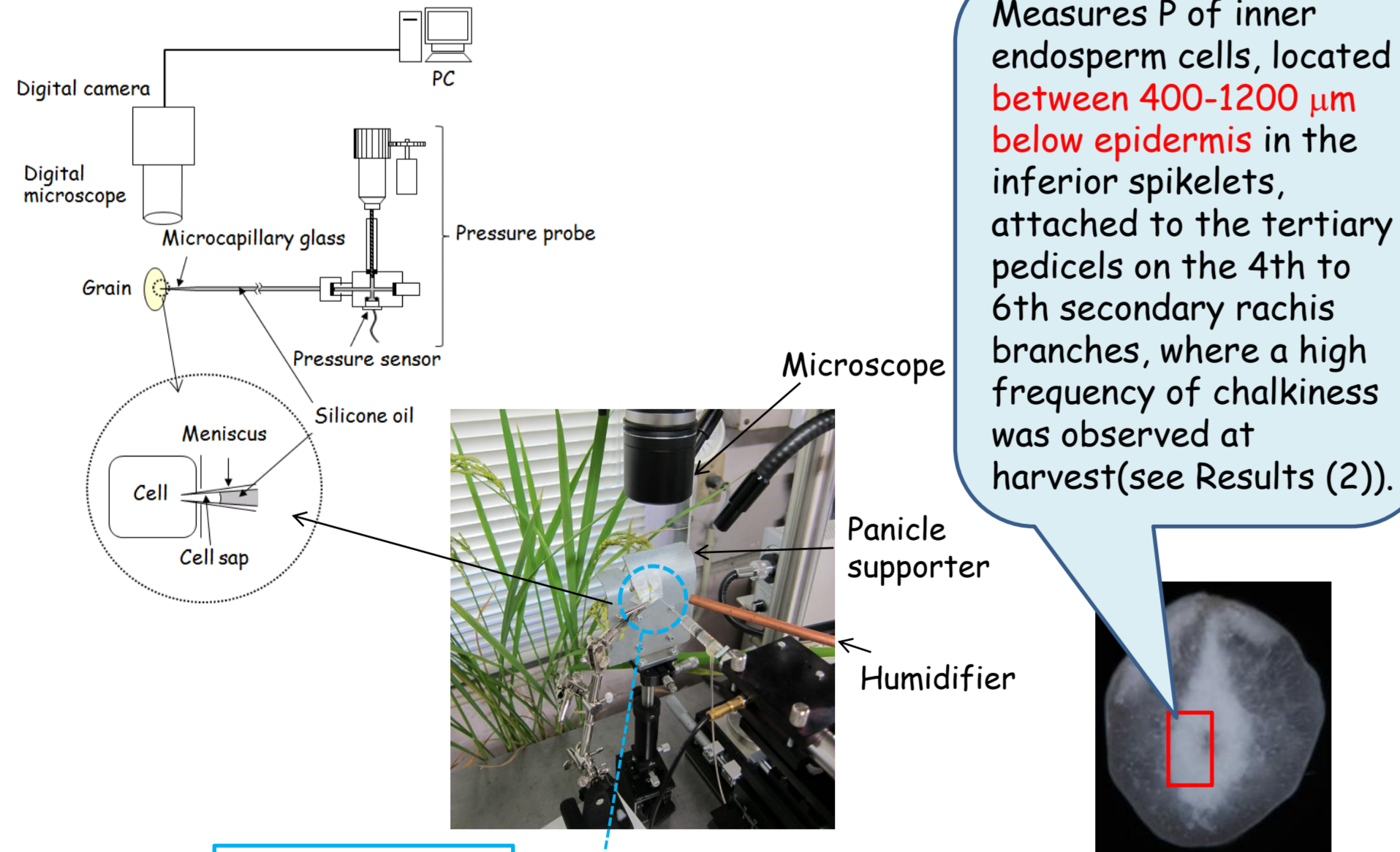
1. Dry wind treatment conducted in a field and growth chamber in ripening. For the field experiment, the effect of VPD on rice appearance was examined (see Result (1))

For the growth chamber experiment (see below), the low light intensity followed by dry wind treatment was conducted for 24 h on the 15th day after heading.



Conducted the following experiments

2. *In situ* P assay in the endosperms with a cell pressure probe (see Results (4)).



Inserted a probe tip into the grain

3. Water potential measurements with a pump-up pressure chamber (PMS Instrument Company, Albany, OR) The kernel water potentials were also determined by psychrometry after determining panicle water potentials (see Results (3)).

4. Photosynthesis measured with a LI-Cor LI-6400 photosynthesis meter (Results not shown).

5. Rice appearance of dehulled grains evaluated with rice analyzers (RGQI 10A and 20A, Satake Co. Ltd.) (see Results (1) and (2)).

Results

(1) Field appearance data indicated that the 24h dry wind treatment (+High VPD wind) substantially decreased perfect rice by increasing ring-shaped chalkiness (MWR) in chalky kernels with no change in other kernels.

Effects on appearance of brown rice treated with two different VPDs of 24 h wind treatment in 2009. The percentage of perfect (translucent), chalky, ring-shaped chalky kernels in chalky kernels, other kernels is shown.

Treatments [†]	Appearance of brown rice [‡]			
	Perfect kernels	Chalky kernels	MWR [§]	Other kernels [§]
No-wind	40.9a [#]	38.2c	28.1b	20.9a
+ Low VPD wind	32.7b	46.0b	32.1b	21.3a
+ High VPD wind	21.7c	59.9a	46.5a	18.5a

[†]Plants were subjected to shade prior to the wind treatments.
[‡]Values were obtained from a rice analyzer with >1.8 mm thickness of grains. Data indicate means of 6 replications taken from 1000 grains per stock with 6 stocks on the second row in each treatment.
[§]Other kernels include damaged, abortive, and colored kernels.
[§]Milky white rice (MWR) includes mostly ring-shaped chalky kernels with white-core and opaque-rice kernels.
[#]Different letters include a significant difference (P<0.05) between means (Tukey's HSD).

The value, 46.5% observed in the ring-shaped chalky rice was almost similar to 2007.

(2) Ring-shaped chalky (MWR) kernel increased by dry wind treatment (+Wind), although there was no difference on the final kernel weight with similar ripened kernels among treatments.

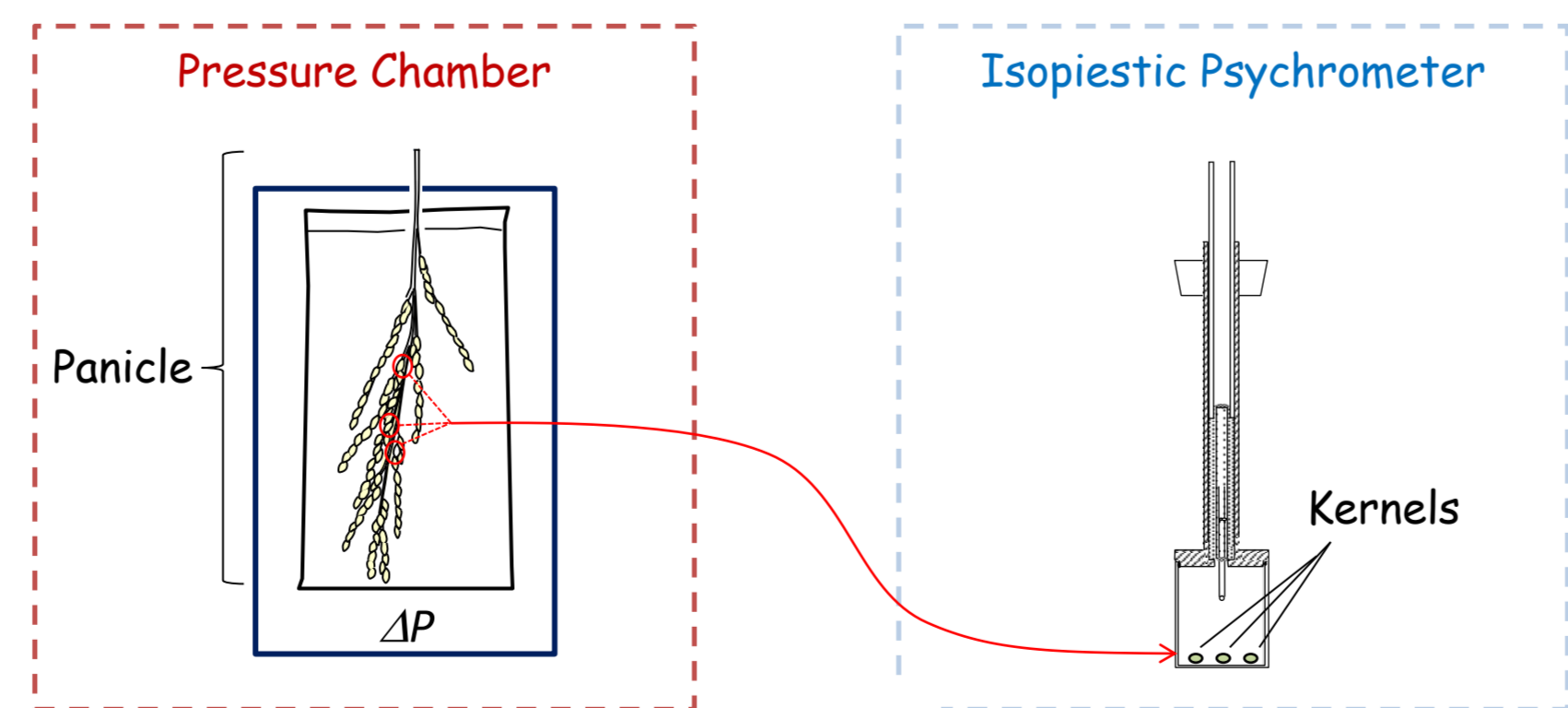
Ripening index, appearance, and grain weight of brown rice grown under 24 h dry wind treatment after low light intensity in growth chambers (see Materials and Methods). The percentage of perfect (translucent) kernels, ring-shaped chalky kernels in chalky kernels, other kernels is shown.

Treatments [†]	Ripened kernels	Appearance of brown rice [‡]				Grain weight [§]
		Perfect kernels	Chalky kernels	MWR [#]	Other kernels [§]	
-Wind	92.4	37.0	37.1	15.2	27.1	24.7
+Wind	90.8	15.8	63.7	34.9	19.9	24.7
Significance ^{††}	NS	***	***	***	**	NS

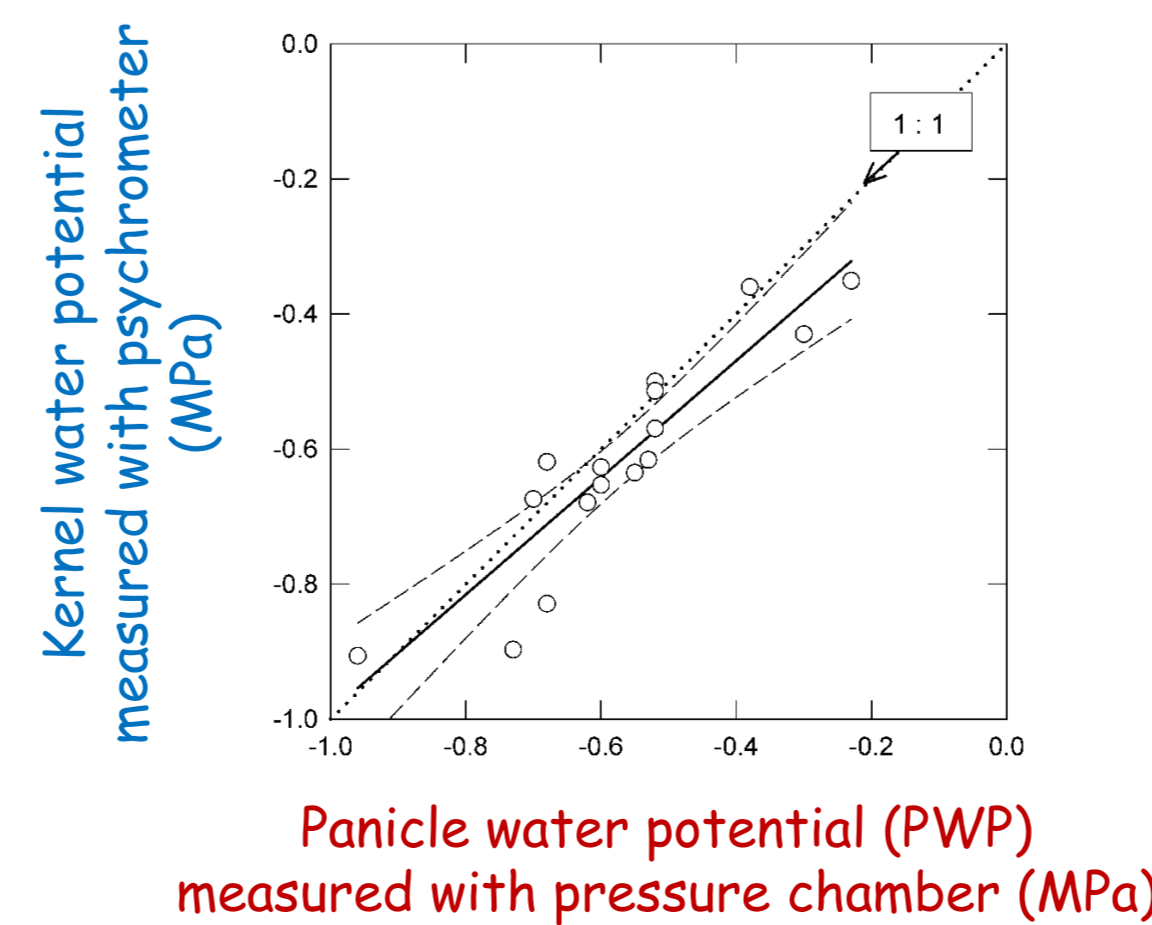
[†]Plants were subjected to low light intensities prior to the wind treatment.
[‡]Values were obtained from a rice analyzer with >1.8 mm thickness of grains. The data indicated means of the pooled brown rice from 5 plants with 4 replications.
[§]Other kernels include damaged, abortive, and colored kernels.
[§]Grain weight is indicated by 15% moisture content.
[#]Milky white rice (MWR) includes mostly ring-shaped chalky kernels with white-core and opaque-rice kernels.
^{††}Significant difference at P = 0.01, and 0.005 is indicated by ** and ***, respectively. NS indicates no statistical significant difference (P <0.05). (Student's t-test, P<0.05).

The largest numbers of ring-shaped chalky kernels were also observed at the position, where cell P measurement was conducted (not shown).

(3) Panicle water potential (PWP) measured with a pressure chamber was highly correlated with the kernel water potential determined by psychrometry at the developmental stage.



The kernels attached to the same position, where the pressure probe measurement was conducted, were collected to determine the tissue-averaged water potentials by using the isopiestic psychrometer after determining the panicle water potential.

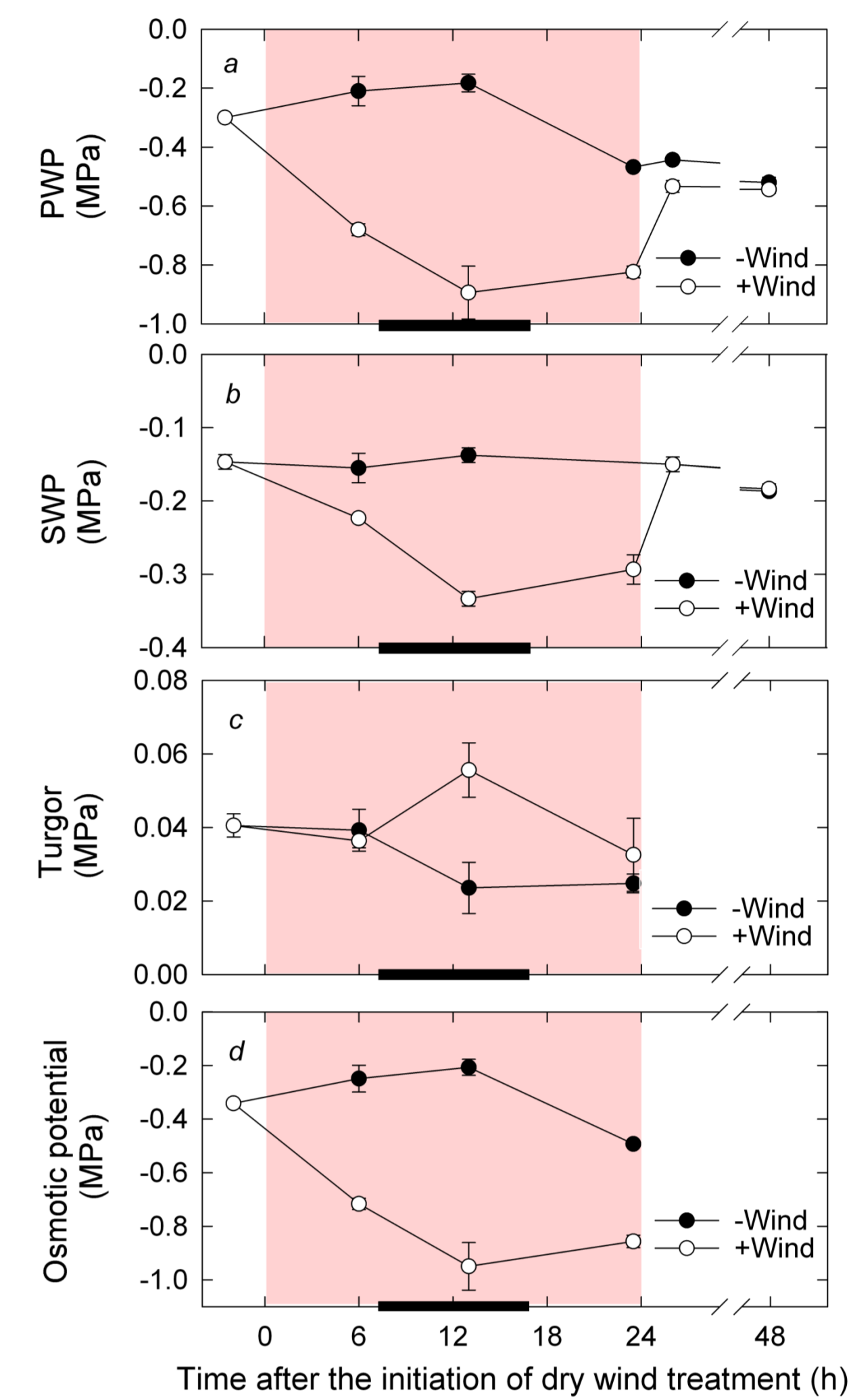


(4) Osmotic adjustment in the endosperm cells occurred at low water potentials prior to chalky formation.

The lowered panicle and stem water potentials indicated that plants were temporarily imposed to water deficit under the dry wind condition (a and b). In addition, turgor maintenance was observed at low water potentials under the dry wind condition (c).

By using the relationship shown in Results (3), the osmotic potential in the endosperms was calculated. The osmotic potential was also declined at low water potential (d), indicating that the cells were osmotically adjusted under the dry wind condition.

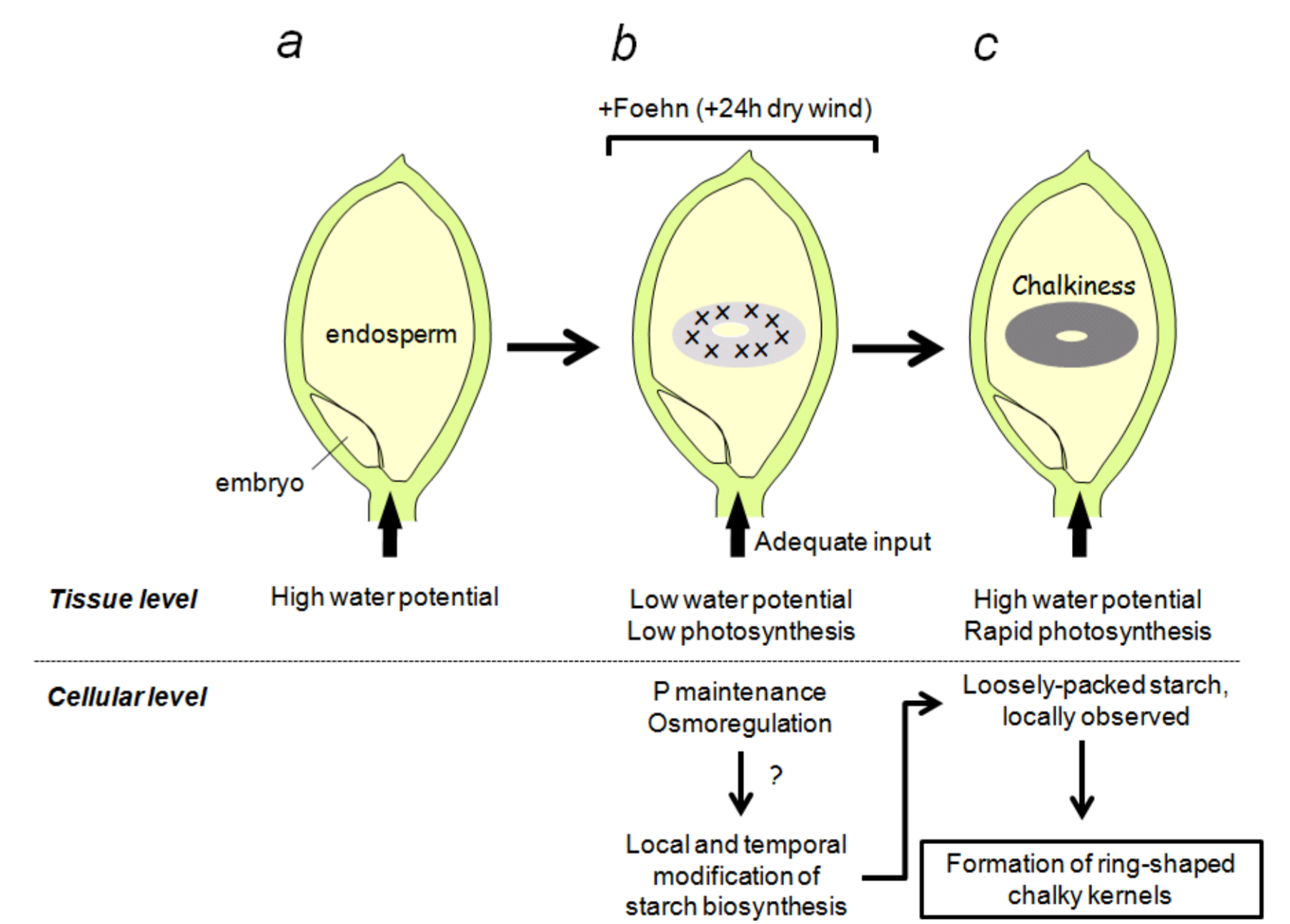
Figure. Changes in the panicle water potential (PWP) (a), stem water potential (SWP) (b), endosperm cell turgor (c), and the endosperm osmotic potential indirectly calculated (d) in developing rice kernels under dry wind conditions. Opened and closed symbols indicate dry wind treatment (+Wind) and control (-Wind), respectively.



Conclusion

- Direct measurements of the water status on endosperm cells indicated that osmotic adjustment in the endosperm cells occurred at low water potentials prior to chalky formation, but with no reduction of ripening index and the final kernel weight.
- Cell P was maintained at low water potentials under dry wind condition, which probably sustained the kernel development by osmotic adjustment.
- Since osmotic adjustment preceded to the chalky formation, osmotic adjustment is believed to have had a role in inhibiting starch accumulation at the temporal water deficit by foehn to increase the ring-shaped chalky kernel.

Schematic for the process of ring-shaped chalkiness formation in developing kernels at low water potential under dry wind condition.



a, Assimilates are delivered to the kernels to be either stored as starch around in endosperm cells or used in developing structures. b, Under the lowered water potentials and partially declined photosynthesis because of the partial stomatal closure under dry wind condition, endosperm maintains P by osmoregulation. c, After dry wind was stopped, water potential and photosynthesis both recovered, although the ring-shaped chalkiness in kernels was formed because of the loosely-packing of starch in the endosperm cells.

Acknowledgement

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