Jpn. J. Crop Sci. 66(3): 449—455 (1997)

High Temperature-Induced Spikelet Sterility of Japonica Rice at Flowering in Relation to Air Temperature, Humidity and Wind Velocity Conditions

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Received May 21, 1996

Abstract: Although the impact of anticipated global warming on regional rice production merits intensive investigation, quantitative information regarding the effects of high temperatures on japonica rice is limited. The purpose of this study was to clarify the critical temperature for high temperature-induced spikelet sterility at the time of flowering in japonica rices and how it is affected by humidity and wind velocity. This research consisted of two experiments. In the first experiment, we examined the response of the varieties 'Akihikari' and 'Koshihikari' to high temperatures at the time of flowering. Under a six-hour high-temperature treatment of panicles for eight days at flowering, the critical temperatures to induce 50% sterility were estimated to be about 36.5°C for Akihikari and 38.5°C for Koshihikari. The major cause of the cultivar difference was attributed to the difference in the number of pollen grains shed on stigma. In the second experiment, we examined the effects of humidity and wind velocity upon the high temperature-induced spikelet sterility of Akihikari. The fertility of spikelets flowered at 37.5°C was highest at 45% relative humidity (R.H.) followed by that at 60% R.H., and lowest at 80% R.H. Wind velocity above 0.85m s^{-1} drastically decreased spikelet fertility at 37.5°C, mainly through reduction of the pollen grain number shed on stigma.
Key words: Air humidity, Cultivar difference, Flowering stage, High temperature, Japonica rice, Pollination, Spikelet sterility, Wind velocity.

日本稲における開花期の高温による不稔の発生と気温および湿度・風速条件との関係：松井　勤・大政謙次*・堀江　武**（京都大学農学部附属農場・*国立環境研究所・**京都大学農学部）

要　旨：日本稲の高温不稔の発生条件を知るために，開花期の昼温が日本型の水稲品種アキヒカリ，およびコシヒカリの受精に及ぼす影響および開花期の昼間の湿度・風速が高温条件下でアキヒカリの稔実に及ぼす影響を明らかにした．昼間6時間の高温処理で50%の不稔を生じる気温はアキヒカリで36.5°C，コシヒカリで38.5°Cと品種間に約2°Cの差がみられた．コシヒカリは高温条件下で柱頭に多くの花粉を付着させる能力をもち，このことがコシヒカリの高温耐性を高めていた．一方，昼温37.5°Cの条件下で，風速0.85 m s^{-1}以上の風は50%も稔実率を低下させることが示された．また，相対湿度は45%～80%の範囲で高いほど稔実率は低下した．高湿度や風は柱頭に付着する花粉粒数の減少を通じて受精率を低下させていた．
キーワード：開花期，高温，湿度，受粉，日本稲，品種間差，風速，不稔．

Recently, it has been predicted by the simulation model that the anticipated global warming of the future would increase the instability of rice yield in southern Japan, mainly through increased probability of high temperature-induced spikelet sterility in japonica rice[3].

Satake and Yoshida studied the problem of high temperature-induced sterility in indica rice[12]. The findings were as follows: (1) high temperature during anthesis time was most detrimental to spikelet fertility; (2) the difference in spikelet sensitivity to high temperature between a susceptible and a tolerant indica rice cultivar was about 4°C; and (3) the major causes of high temperature-induced sterility were attributed to disturbed pollen shedding and decreased viability of pollen grains, resulting in a decreased number of germinated pollen grains on the stigma.

However, only a few studies have so far been conducted on high temperature-induced spikelet sterility in japonica rice cultivars[3,5,6,8,13], because it has scarcely had effect on rice yield in Japan. To validate the prediction by simulation model, precise data are necessary on japonica cultivar differences in high temperature-induced sterility, and on the effects of related environmental factors, such as temperature, humidity and wind velocity on sterility. This study had three objectives : To quantify the critical temperatures for high temperature spikelet sterility in japonica rice, to clarify the effects of air humidity and wind velocity on high temperature-induced spikelet sterility, and to explore the possible mechanisms of the high temperature spikelet sterility in rice.

Materials and methods

The investigation consisted of two experiments. Experiment I dealt with the temperature effect on spikelet fertility and its cultivar difference, and Experiment II with the effect of air humidity and wind velocity on high temperature-induced rice spikelet sterility.

Experiment I

Two japonica rice cultivars, Akihikari and Koshihikari, were used for this experiment. Twenty seedlings each at 5.2 leaf stage were transplanted in a circular pattern into 1/5000a Wagner pots on June 19, 1994 for Koshihikari, and on July 10, 1994 for Akihikari, and were grown in submerged conditions. Each pot was provided with 0.2g N, and 0.6g each of P_2O_5 and K_2O as basal fertilizer and 0.4g N as top-dressing at 20 days before heading. Tillers were removed during the vegetative stage as they appeared. The fertility percentage of materials which flowered under outdoor conditions (middle August) was 94.6% for Akihikari and 94.2% for Koshihikari. Sunlighted phytotrons at the National Institute for Environmental Studies (NIES) were used for high temperature treatments on rice. Plants at middle heading stage were subjected to high temperature treatments of 36.5°C, 38.0°C, and 39.5°C for 6hours (10 : 00 − 16 : 00) for 8 consecutive days in mid-August. Night temperature (17 : 00 − 9 : 00) was fixed at 26°C for all treatments. Air temperature and humidity were gradually changed from 9 : 00 to 10 : 00 and from 16 : 00 to 17 : 00. Three pots were used for each treatment. A number of spikelets (32 to 49 spikelets per treatment) that flowered on the third day after starting high temperature treatment were sampled for microscopic observation of pollen in each treatment. The total number of pollen grains and the number of germinated pollen grains on each stigma of the sample spikelets were counted under a microscope by staining with cotton blue. The germination percentage of pollen grains in a treatment was calculated as the average of germination percentages of pollen grains on each stigma. Percent fertility of 15 to 30 panicles in each treatment that headed during the first three days of treatment were examined at maturity.

Experiment II

To study the effect of humidity and wind velocity on the spikelet fertility that flowered under high temperature conditions, 20 pregerminated seeds of the japonica rice cultivar Akihikari were sown in a circular pattern in 1/5000a wagner pots, and were grown in a green house. Temperature was kept at 25°C and day length for 14 hours by supplementary illumination. Each pot was provided with 0.5 g each of N, P_2O_5, K_2O. Tillers were removed as described in Experiment I. The Natural Environment Simulator of NIES[1)], a facility for simultaneous control of air temperature, humidity, wind velocity and illumination, was used for temperature, humidity and wind treatments. Plants at middle heading stage were used. Plants were subjected to humidity treatments of three levels (45%, 60%, and 75% relative humidity at wind velocity 0.5m s^{-1}), or to wind treatment of three levels (0.2m s^{-1}, 0.85m s^{-1} and 1.5m s^{-1} at 60% relative humidity) from 10 : 00 to 16 : 00. Day temperature was 37.5°C (10 : 00 − 16 : 00) and night temperature was 25°C (18 : 00 − 9 : 00, 17 : 00 − 18 : 00). Relative humidity and wind velocity at night was fixed at 75% and 0.5m s^{-1}, respectively. Air temperature, humidity and wind velocity were gradually changed from 9 : 00 to 10 : 00 and from 16 : 00 to 17 : 00. Illumination was 30000 Lux (5 : 00 − 18 : 00). Three to five pots were used for each treatment. Spikelets that flowered from 12 : 00 to 13 : 00 on the treatment day were marked on the glume surface with acrylic paint. A part of these marked spikelets (34 to 96 per treatment) were sampled at 16 : 00 on the treatment day for microscopic observation. The total number of pollen grains and the number of germinated pollen grains on each stigma of the sampled spikelets were counted under a microscope by staining with cotton blue. The germination percentage of pollen grains was calculated as the average of germination percentages of pollen grains on each stigma. Remaining marked spikelets (152 to 250 spikelets per treatment) were subjected to fertility investigation at maturity.

Results

1. Cultivar difference in high temperature effect on spikelet fertility

Fig. 1 shows percent fertility of spikelets on the panicles which headed during the first three days of the treatment. The temperature at which the percent spikelet fertility decreased to 50% was about 36.0—36.5°C for Akihikari and 38.0—38.5°C for Koshihikari. Thus, the difference in spikelet sensitivity to high temperature sterility was about 2°C between the two varieties. Spikelet fertility increased linearly with the number of germinated pollen grains per stigma in both

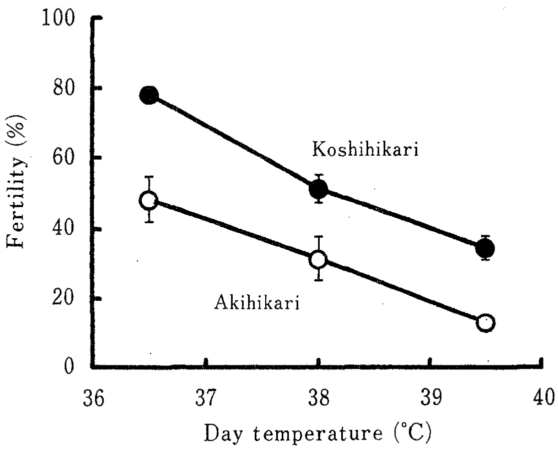

Fig. 1. Percent fertility of rice spikelets flowering at different day time temperatures.
Day time temperature was maintained for 6 hours (10 : 00—16 : 00) and night-time temperature was fixed at 26°C in each treatment. Vertical bars indicate the standard errors of means (n=3).

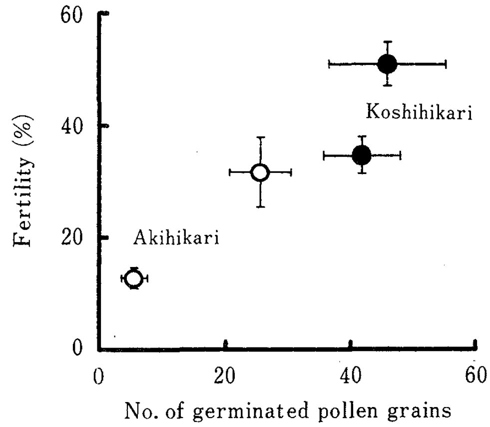

Fig. 2. Relationship between fertility and the number of germinated pollen grains per stigma of spikelets that flowered on the third day after the initiation of high temperature treatments.
High temperature was maintained for 6 hours (10 : 00—16 : 00). Night-time temperature was fixed at 26°C in all treatments. Bars indicate the standard errors.

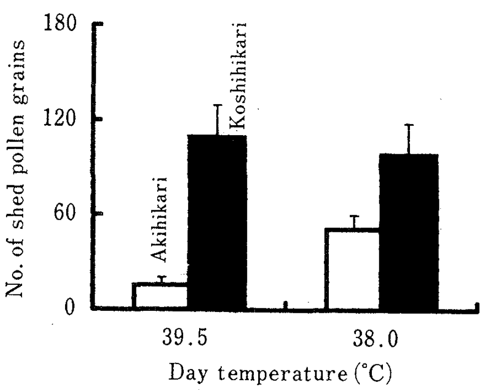

Fig. 3. Comparison of the number of shed pollen grains per stigma of spikelets that flowered on the third day after starting high temperature treatments between the two japonica rice cultivars.
Day time temperature was maintained for 6 hours (10 : 00—16 : 00) and night time temperature was fixed at 26°C in each treatment. Vertical bars indicate the standard errors of means (n=3).

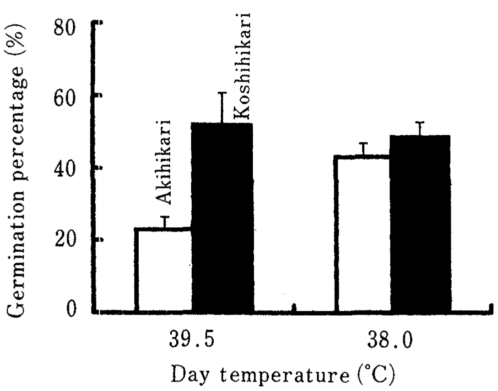

Fig. 4. Comparison of the germination percentage of pollen grains on stigmas of spikelets that flowered on the third day after starting high temperature treatments between the two japonica rice cultivars.
Day time temperature was maintained for 6 hours (10 : 00—16 : 00) and night time temperature was fixed at 26°C in each treatment. Vertical bars indicate the standard errors of means (n=3).

varieties (Fig. 2). Fig. 3 shows the numbers of shed pollen grains on each spikelet stigma of both varieties that flowered under different temperature conditions. The numbers of shed pollen grains on each stigma in Akihikari were about 50% at 38.5°C and about 20% at 39.5 °C of those in Koshihikari. To clarify the effect of high temperature on pollen activity, the germination percentage of pollen grain on each stigma was calculated (Fig. 4). The germination percentage in Akihikari was similar to that in Koshihikari at 38°C and about 50% of that in Koshihikari. It follows from these results that the primary cause for the difference in the number of germinated pollen grains between the two cultivars was the difference in number of shed pollen grains on a stigma.

2. Effect of air humidity and wind velocity on high temperature-induced spikelet sterility

Fig. 5-a shows the effect of air humidity (45−75% R.H.) at flowering on the fertility percentage of Akihikari under a 37.5/25.0°C of day/night temperature regime. As the relative humidity increased from 45% to 70%, percent fertility decreased. Fig. 6-a depicts the effect of wind velocity at flowering on percent fertility of spikelets of Akihikari under the same temperature conditions. Fertility percentage rapidly dropped as wind velocity exceeded ca. 0.5m s^{-1}. Fertility percentage of spikelets was closely related to the number of germinated pollen grains per stigma in both treatments as shown in Fig. 7.

In the humidity treatment, the number of

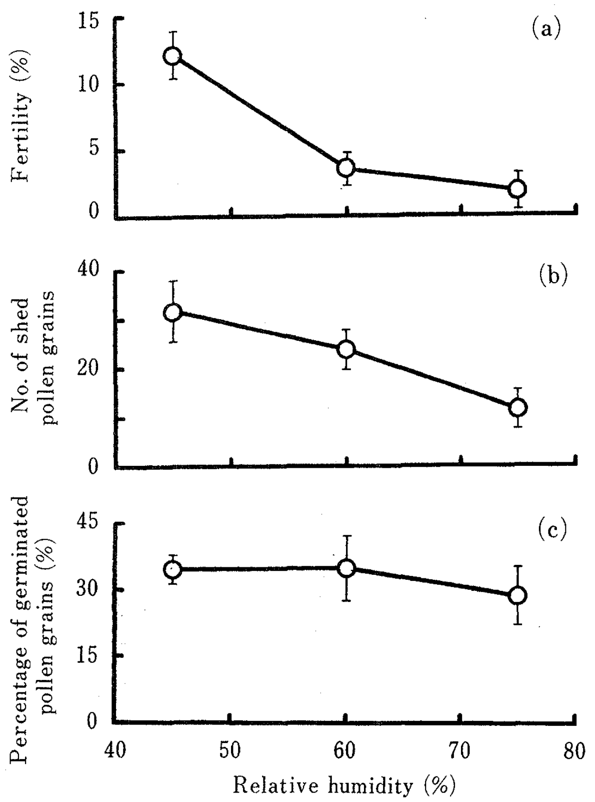

Fig. 5. Fertility (a), number of shed pollen grains per stigma (b) and percentage of germinated pollen grains on stigmas (c) of spikelets flowering at different day time humidity levels (cultivar : Akihikari).
Day and night time temperatures were 37.5°C and 26.0°C, respectively. Wind velocity and night-time humidity were fixed at 0.5m s^{-1} and 75%, respectively. Vertical bars indicate the standard errors of means (n=3).

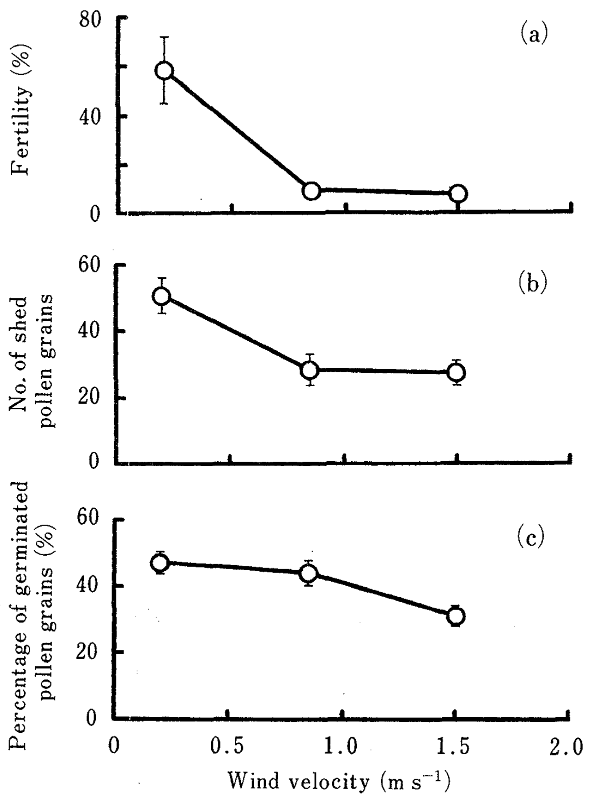

Fig. 6. Fertility (a), number of shed pollen grains per stigma (b) and percentage of germinated pollen grains on stigmas (c) of spikelets flowering at different day time wind velocities (cultivar : Akihikari).
Temperature and humidity were kept at 37.5 °C and 60% during day time and at 26 °C and 75% during night time, respectively, in all treatments. Night-wind velocity was 0.5m s^{-1}. Vertical bars indicate the standard errors of means (n=5).

pollen grains on each stigma decreased gradually as the relative humidity increased from 45% to 75%, while the germination percentage of pollen grains almost unchanged. In the wind treatment, the number of pollen grains shed on stigma drastically reduced at 0.85m s^{-1} wind velocity, while the germination percentage of pollen grains deposited on stigma was not affected by the wind velocity. These results indicate that the pollination process is more sensitive to humidity and wind than the process of pollen germination under high temperature conditions.

Discussion

It has been shown that about 2°C cultivar difference exists in the sensitivity to high temperature stress (Fig. 1). The 2°C difference in the sensitivity to heat stress has been shown to give an enormous difference in rice yield under predicted global warming conditions for Japan[4]. Therefore, clarification of cultivar difference in the spikelet sensitivity to high temperature-induced sterility is important for impact assessment of global climate change on rice yield, and for breeding of high temperature-tolerant cultivars.

Humidity and wind velocity at flowering were also shown to have significant effects on spikelet fertility under high temperature (Figs. 5-a and 6-a). In particular, even wind velocities below 1m s^{-1} diminished spikelet fertility more than 50% (Fig. 6-a). However, it should be noted that the wind treatments in this experiment were made under a constant velocity throughout the day and differed from natural wind conditions where velocity fluctuations are common. Although this fact necessitates further investigation on the wind effects under natural conditions, it may be concluded that a higher wind speed at flowering of rice under high temperatures has a negative effect on fertility.

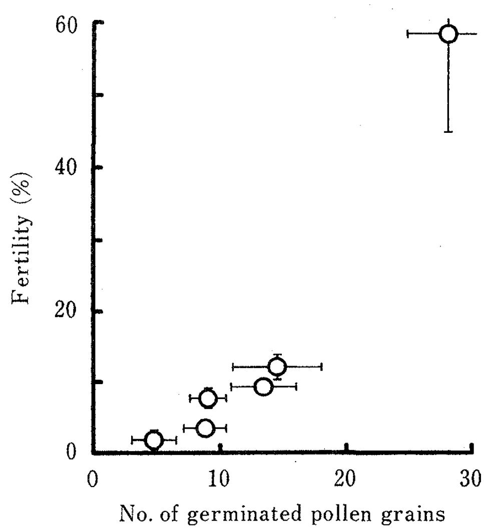

Fig. 7. Relationship between fertility and the number of germinated pollen grains per stigma under different wind and humidity conditions (cultivar : Akihikari).
Day- and night-time temperatures were fixed at 37.5°C and 26.0°C, respectively, in all treatments. Bars indicate the standard errors.

Let us now discuss the mechanisms of the cultivar difference in the high temperature tolerance and the effect of humidity and wind velocity on the spikelet fertility. Satake and Yoshida[12] demonstrated that sound pollen could fertilize under 38°C air temperature condition by artificial pollination. They also found that percentage of spikelets with more than 10 germinated pollen grains on the stigma gave agreement with the percent fertility under different high temperature conditions. Their results indicate that the process of fertilization after pollen germination is considerably tolerant to high temperature. In the current experiments, the number of germinated pollen on a stigma is closely related to the fertility percentage, suggesting that cultivar difference in the percent fertility and the high humidity and wind velocity effects on the fertility were all the result of the difference in the number of germinated pollen grains per stigma (Figs. 2 and 7).

In Experiment I, the primary cause for the difference in number of germinated pollen grains per stigma between Koshihikari and Akihikari was the difference in number of shed pollen on a stigma (Figs. 3 and 4). This result is in accordance with the result for indica rice by Satake and Yoshida[12]. They observed that, in a tolerant cultivar N22, dehiscence of anthers starts immediately after glume opening. We also observed that many anthers of Akihikari remain closed after flowering under high temperature conditions, while those of Koshihikari were empty, indicating that Koshihikari has a tendency to dehisce anther under high temperatures, similarly to N22.

After pollen shedding, the germination percentage determines the number of germinated pollen per stigma. Enomoto et al.[2] reported that the critical high temperature for pollen germination ranged from 41°C to 45°C, indicating that sound pollen on stigma can germinate under considerably high temperatures. These facts imply that the observed decline in the pollen germination percentage in Akihikari under 39.5°C was caused by a reduction of pollen activity before shedding (Fig. 4).

Our results also proved that high humidity disturbs the pollination of japonica rice under high temperature conditions (Fig. 5-b). Nishiyama and Satake[9] referred to the observation that, under severe high temperature stress at flowering, fertility of indica rice was higher in dry air than in humid air conditions. They hypothesized that dry air promotes by desiccation the dehiscence of anthers or curbs extra elongation of filaments under high temperature conditions. Our results were in agreement with these observations and seem to support their hypothesis.

Though wind also desiccates anther or spikelets, above 0.85m s^{-1} it disturbes pollination (Fig. 6-b). Wind disturbance of pollination may be related to the position of the anther at its dehiscence time. It was observed that anther position relative to glume tips at time of dehiscence was higher at higher temperatures. Under such circumstances, a strong wind might blow away substantial numbers of pollen grains and disturb self-pollination. This could explain why spikelets which flowered under high temperature were more sensitive to wind than those under moderate temperature conditions.

The present results indicate that spikelet sterility under high temperature at flowering is not due to injury by simple desiccation, which is contrary to results reported elsewhere. Matsushima et al.[7] showed that low humidity promoted spikelet sterility under high temperature in an experiment of rice cultivation in the Sudan. Roy and Acharya[11] reported that sterility at flowering in India is mainly due to the desiccation of pollen. However, these results were obtained under much drier air conditions than the present experiment. Therefore, it seems that the sterility that occurred in those experiments might be caused by desiccation rather than by high temperature.

The conclusion from the above arguments is: (1) Flowering time temperature above 36 °C induces spikelet sterility of japonica rice and a considerably large cultivar difference exists in spikelet sensitivity to high temperature damage. The critical air temperatures at which spikelet fertility percentage reaches 50% are estimated to be 36.5°C for Akihikari and 38.5°C for Koshihikari; (2) The primary cause for the cultivar difference in the high temperature tolerance at flowering was the number of pollen grains shed on a stigma; (3) Higher air humidity and wind velocity increase spikelet sterility induced by high temperatures mainly through disturbance of pollination; and (4) high temperature sterility at flowering is not due to a simple desiccation injury.

Anther dehiscence of rice plant has been perceived as a simple desiccatory process. If so, high temperature would promote a dehiscence of anther and increase the number of shed pollen on stigma. It is therefore difficult to explain the mechanism through which anther dehiscence is disturbed by high temperature on the basis of this hypothesis. Reconsideration of the anther dehiscence process seems necessary for explaining this mechanism in relation to humidity and wind velocity, which will also contribute to explaining the cultivar difference in high temperature tolerance in pollination.

Acknowledgment

We thank H.Yabuki and N. Okamoto of Kyoto University for their technical assistance.

References

1. Aiga, I., K. Omasa and S. Matumoto 1982. Phytotron of the National Institute for Environmental Studies and its Energy Supply System. SHASE Journal 56 : 741—751*.
2. Enomoto, N., I. Yamada and K. Hozumi 1956. On the artificial germination of pollen in rice plants. IV. Temperature limits of pollen germination in rice varieties. Proc. Crop Sci. Soc. Japan 25 : 69*.
3. Horie, T. 1993. Predicting the effects of climate variation and elevated CO_2 on rice yield in Japan. J. Agr. Met. 48 : 567—574.
4. ————, T. Matsui, H. Nakagawa and K. Omasa 1996. Effect of Elevated CO_2 and Global Climate Change on Rice Yield in Japan. In Omasa, K. et al. eds., Climate Change and Plant

in East Asia. Springer Verlag, Tokyo. 39—56.
5. Imaki, T., S. Tokunaga and N. Obara 1987. High temperature sterility of rice spikelets at flowering in relation to flowering time. Jpn. J. Crop Sci. 56 (extra issue 2) : 209—210**.
6. Matsui, T. and T. Horie 1992. Effect of elevated CO_2 and high temperature on growth and yield of rice. 2. Sensitive period and pollen germination rate in high temperature sterility of rice spikelets at flowering. Jpn. J. Crop Sci. 61 (extra issue 1) : 148—149**.
7. Matsushima, S., H. Ikewada, A. Maeda, S. Honda and H. Niki 1982. Studies on rice cultivation in the tropics. 1. Yielding and ripening responses of the rice plant to the extremely hot and dry climate in Sudan. Jpn. J. Trop. Agr. 26 : 19—25.
8. Nabeshima, M., M. Ebata and M. Ishikawa 1988. Studies on the high temperature and dry wind injuries in flowering rice plant. Fertilization injury induced by high temperature. Rep. Tokai Br. Crop Sci. Japan. 105 : 1—2***.
9. Nishiyama, I. and T. Satake 1981. High temperature damage in rice plant. Jpn. J. Trop. Agr. 25 : 14—19***.
10. Osada, A., V. Sasiprapa, M. Rahong, S. Dhammanuvong and H. Chakrabandho 1973. Abnormal occurrence of empty grains of indica rice plants in the dry, hot season in Thailand. Proc. Crop. Sci. Soc. Japan 42 : 103—109.
11. Roy, A. and N. Acharya 1981. Temperature tolerance. Intern. Rice Res. Newsl. 6(4) : 9—10.
12. Satake, T. and S. Yoshida 1978. High temperature-induced sterility in indica rice at flowering. Jpn. J. Crop Sci. 47 : 6—10.
13. Sato, K., K. Inaba and M. Tazawa 1973. High temperature injury of ripening in rice plant. 1. The effects of high temperature treatments at different stages of panicle development on the ripening. Proc. Crop Sci. Soc. Japan. 42 : 207—213*.

* In Japanese with English summary.
** Translated from Japanese by the present authors.
*** In Japanese.