Bull.Fac. Life Env. Sci. Shimane Univ.,　6：23－29, December 20, 2001

# Quantitative Relation of Spikelet Degeneration with Available Carbohydrate in the Mid-Reproductive Stage

Kazuhiro KOBAYASI, Kazuyoshi YAMANE and Tadashi IMAKI

Faculty of Life and Environmental Science, Shimane University, Matsue　690－8504,　Japan

**Abstract**　A considerable percentage of spikelets degenerates in the mid-reproductive stage. To quantify this percentage, shading and thinning treatments were carried out to change the available carbohydrate in plants. Dry matter production and the content of non-structural carbohydrate（NSC）were changed by three shading intensities and one thinning treatment of alternate rows during the mid-reproductive stage. The numbers of differentiated and degenerated spikelets were counted at heading. Shading increased the spikelet degeneration percentage and thinning decreased it by changing shoot dry matter production and stem NSC content. Spikelet degeneration percentage was negatively correlated with both the dry matter production per differentiated spikelet and the NSC amount per differentiated spikelet. We concluded that available carbohydrate per differentiated spikelet determines the number of degenerated spikelets through spikelet competition. Competition for assimilates among spikelets implies that available carbohydrate partitioning would not be optimal for spikelet production and that a part of the available carbohydrate would be used wastefully for degenerated spikelets.

Key words: Degenerated spikelet, Dry matter production, Non-structural carbohydrate, Reproductive stage, Rice.

## Introduction

Spikelet number per unit area is a strong determinant of rice yield（Senanayake et al. 1996）. Final spikelet number is the difference between the numbers of differentiated and degenerated spikelets（Matsushima, 1957）. A large number of spikelets degenerate in the mid-reproductive stage that centers on the meiosis stage. Spikelet number per panicle increases during the spikelet differentiation stage, and then decreases mainly around the meiosis stage（Matsushima, 1957； Senanayake et al., 1991）. The percentage of degenerated spikelets（spikelet degeneration percentage）is highly correlated with the number of differentiated spikelets, and averages 20－25%（Kobayasi, 2000）. However, higher spikelet degeneration percentages, such as 40－50%, have been reported（Matsushima, 1959）. These reports suggest that it is possible to increase final spikelet number by decreasing spikelet degeneration percentage

Shading（Matsushima, 1957）, insufficient nitrogen（N）nutrition（Matsushima, 1957；Senanayake et al. 1996）, and removal of leaf blades or roots（Matsushima, 1957）increase the number of degenerated spikelets. Shading during the mid-reproductive stage seriously decreases final spikelets per panicle to nearly half that of control（Matsushima, 1957）. In Japan, N fertilizer is commonly applied at the mid-reproductive stage to decrease spikelet degeneration. Removal of leaf blades or roots greatly increases spikelet degeneration percentage to nearly 40%（Matsushima, 1957）. Probably reduction in available carbohydrate due to suppressed photosynthesis is the cause of spikelet degeneration by shading, insufficient N, and leaf or root removal. This implies that available carbohydrate for spikelet survival might be the key factor in evaluating the number of degenerated spikelets.

To quantify the number of degenerated spikelets, we formed two hypotheses based on different mechanisms in determining spikelet degeneration and survival. One hypothesis（Hypothesis I）is that the amount of available carbohydrate in the mid-reproductive stage proportionally determines final spikelet number. This hypothesis emphasizes the optimization of carbohydrate partitioning among organs. In wheat, the number of kernels per $m^2$ was related to photothermal quotient, which is calculated from solar radiation and mean temperature in the 30 days preceding anthesis and related to dry matter accumulation during the reproductive stage（Fischer, 1985）. In rice, spikelet number per unit area was related to dry weight and

N concentration, so that soluble carbohydrate content of the crop was related to the determination of spikelet number（Hasegawa et al., 1994）. The other hypothesis（Hypothesis II）is that available carbohydrate per differentiated spikelet determines spikelet degeneration percentage by determining how many differentiated spikelets can survive. This hypothesis emphasizes competition among differentiated spikelets. Wada（1969）related the number of degenerated spikelets per $m^2$ to dry matter production per differentiated spikelet from the late stage of spikelet differentiation to heading.

There are several possible indicators in evaluation of available carbohydrate for final spikelets, but in this experiment, two values were used as indicators of available carbohydrate. One was dry matter production during the mid-reproductive stage and the other was the amount of non-structural carbohydrate（NSC）at the meiosis stage. In the mid-reproductive stage, panicles and other organs are growing vigorously and will need a lot of assimilates, so that dry matter increase will be a good estimator of carbohydrate available for spikelet development. Non-structural carbohydrate at the meiosis stage will be another good estimator in evaluating available carbohydrate because NSC is an essential substrate for developing reproductive organs. In some deterministic models, the rate of carbohydrate flow has been used as a determining factor in organ proliferation（Dayan et al., 1981）.

Our objectives are to quantify the relationship between available carbohydrate and spikelet degeneration and to examine which hypothesis is valid in quantification of spikelet degeneration. In this study, dry matter production and NSC content were changed by shading and thinning in the mid-reproductive stage. At heading, the number of degenerated spikelets was counted. The relationships of spikelet degeneration to shoot dry matter production and NSC content in the plants were examined.

## Materials and Methods

### 1. Plant materials and shading and thinning treatments

Two cultivars of japonica rice（Koshihikari and Nipponbare）were used. The experiment was conducted in the experimental paddy field of Shimane University in Matsue, Shimane Prefecture, Japan （35°27’N latitude, 133°43’E longitude, and 17m altitude）. The soil was alluvial and sandy clay. Thirty-day-old seedlings grown in nursery boxes were transplanted on May 15, 1996. Planting density was 22.2 hills/$m^2$（two seedlings per hill; hill spacing of 15cm and row spacing of 30cm）. Basal application and top-dressing were carried out according to the standard for plain fields in Shimane Prefecture（Table 1）. The experimental design was a split plot design with two replicates. The area of each plot ranged from 4$m^2$（shaded）to 20$m^2$（thinned）.

To change dry matter production and NSC content, shading and thinning treatments were conducted. The treatments began at the beginning of the meiosis stage（Koshihikari, July 23；Nipponbare, August 1）and lasted for seven days. Three shading intensities, 34%（low shading）, 58%（middle shading）, and 82%（high shading）, were carried out with combinations of two types of shade cloth（34 and 58% shading）. The thinning was achieved by the removal of alternate rows using a hand sickle（thinning percentage 50%）, with rice plants cut about 5cm above the soil surface.

**Table 1.** The amount（g/$m^2$）and the time of basal application and top-dressing in two cultivars（Koshihikari and Nipponbare）

| | Basal | Tillering* | Panicle Formation** | Booting*** |
|---|---|---|---|---|
| Koshihikari | | | | |
| N | 3 | 0 | 1.5 | 1.5 |
| $P_2O_5$ | 3 | 0 | 0 | 0 |
| $K_2O$ | 3 | 0 | 3 | 0 |
| Nipponbare | | | | |
| N | 4 | 2.5 | 2.5 | 2.5 |
| $P_2O_5$ | 4 | 0 | 0 | 0 |
| $K_2O$ | 4 | 2.5 | 5 | 0 |

* Active tillering stage（Nipponbare, June 1）.
** Panicle formation stage（Koshihikari, July 18；Nipponbare, July 18）.
*** Booting stage（Koshihikari, July 30；Nipponbare, July 28）.

### 2. Measurement of NSC and N content

Eight hills per replicate were sampled at the beginning and at the end of the treatments. Plant length, leaf area and tiller number were measured. After removing the roots, the plants were divided into leaf blades（leaves）and the rest（stems）. Each part was dried at 80℃ for more than 48h to measure the dry weight for each part. Shoot dry weight used in this experiment was the sum of the dry weights of leaves and stems. The samples were ground and then used for chemical analysis for N and NSC determination. Nitrogen concentration in each plant part was determined by the semi-micro Kjeldahl method. Shoot N content was determined as the sum of leaf and stem N content.

The concentration of NSC in the stems was measured as the sum of the concentrations of soluble sugar and starch. Soluble sugar was extracted with 80% ethanol, and its content was measured by the anthron sulfuric acid method. The rest of the samples from which soluble sugar was extracted were hydrolyzed by amyloglucosidase, and starch content was measured by Food Analysis Starch（Boehringer Mannheim Co., Ltd., Mannheim, Germany）.

### ３．Counting the number of differentiated spikelets

The number of differentiated spikelets was defined as the sum of the number of final spikelets and the number of degenerated spikelets. In this study, final spikelets were defined as surviving spikelets used for a yield component. Panicles of eight hills per replicate were sampled a few days before heading. Degenerated spikelets were determined by counting vestiges of degenerated organs on the panicles with the naked eyes or a binocular microscope（Matsushima, 1957）. Spikelet degeneration percentage was defined as the ratio of the number of degenerated spikelets to that of differentiated spikelets.

## Results

### １．Effects of shading and thinning on number of degenerated spikelets

The number of differentiated spikelets per hill was not changed by shading and thinning treatments in either cultivar because the treatments started after most of the spikelets had differentiated（Table 2）. Koshihikari plants differentiated more spikelets than Nipponbare plants. Shading treatment increased the number of degenerated spikelets in both cultivars（Table 2）. High shading in particular increased the number of degenerated spikelets of Koshihikari and Nipponbare plants by 44% and 42% compared to control, respectively. The thinning treatment decreased the number of degenerated spikelets of Koshihikari and Nipponbare by 7.9% and 37% compared to control, respectively. Final spikelet number per hill in both cultivars was slightly decreased by the shading treatments because of the increased degenerated spikelet number. Spikelet degeneration percentage was also changed by the treatments in accordance with increase in degenerated spikelet number.

### ２．Effects of shading and thinning on shoot dry matter production and distribution

Shading decreased dry matter production of both cultivars during the treatments（Fig. 1）. At the beginning of the treatments, shoot dry weight in Koshihikari rice was 29.80g per hill. After the treatments, control Koshihikari plants gained 2.33g of dry weight per hill. High-shaded Koshihikari gained only 1.53g, and thinned Koshihikari produced 6.49g dry matter during the treatments. The effects of shading and thinning on the dry matter production in Nipponbare rice were similar to those in Koshihikari rice.

In both cultivars, dry weight changes in the stems during the treatments were larger than those in the leaves. The thinning

**Table 2.** The effects of shading and thinning on the numbers of differentiated, degenerated and final spikelets per hill and the concentration and the amount of non-structural carbohydrate in the stems（NSC）.

| Cultivar | Treatment | Differentiated spikelets | Degenerated spikelets | Final spikelets | Spikelet degeneration percentage（%） | NSC concentration （mg/g） | NSC amount （g/hill） |
|---|---|---|---|---|---|---|---|
| Koshihikari | High shading | 1803±176 | 385±64 | 1419±113 | 21.2±1.5 | 49.4±14.7 | 1.00±0.22 |
| | Middle shading | 1859±195 | 272±84 | 1587±111 | 14.3±3.0 | 79.7±18.3 | 1.87±0.30 |
| | Low shading | 1881±22 | 278±33 | 1603±55 | 14.8±1.9 | 132.5±10.7 | 3.88±0.19 |
| | Thinning | 1790±178 | 246±0 | 1543±178 | 13.9±1.4 | 174.5±0.2 | 4.67±0.43 |
| | Control | 1820±336 | 267±86 | 1553±250 | 14.3±2.1 | 147.7±38.8 | 3.39±1.26 |
| Nipponbare | High shading | 1436±167 | 246±116 | 1191±51 | 16.4±6.2 | 56.1±3.2 | 1.40±0.13 |
| | Middle shading | 1313±98 | 127±40 | 1186±58 | 9.5±2.3 | 106.3±7.5 | 3.15±0.04 |
| | Low shading | 1368±128 | 127±46 | 1241±81 | 9.0±2.5 | 125.5±14.1 | 3.85±0.00 |
| | Thinning | 1388±120 | 109±12 | 1279±132 | 8.0±1.5 | 188.9±24.6 | 6.88±0.74 |
| | Control | 1461±167 | 173±75 | 1289±92 | 11.4±3.8 | 147.6±1.2 | 4.44±0.37 |

Mean±S.E.（n=2）.

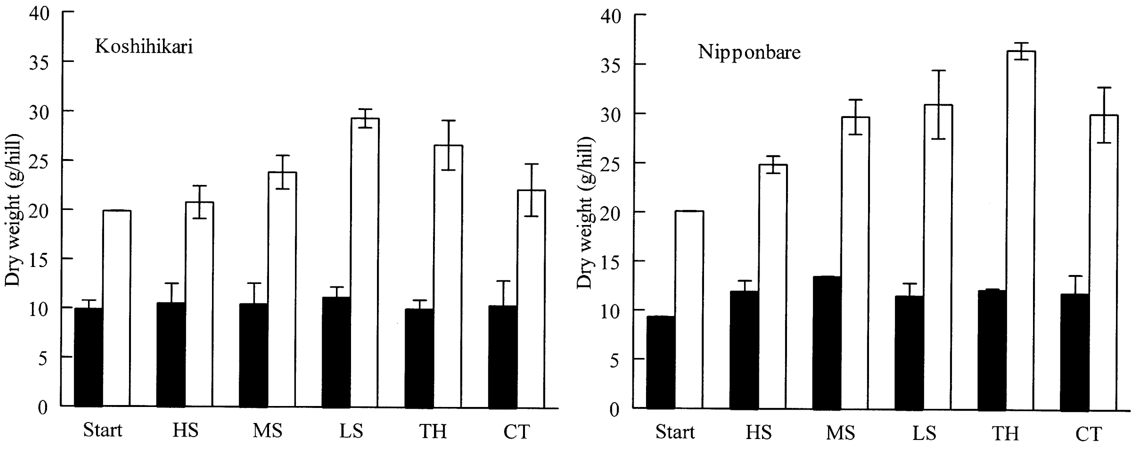

**Fig.** 1.　Dry weight distribution between leaves and stems in Koshihikari（upper）and Nipponbare（lower）.
Leaf weight（black bar）is the weight of leaf blades. Stem weight（white bar）is the weight of the rest of the shoots. Bars indicate standard errors（n =2）.
Start: at the start of the treatments, HS: high shading（82% shading）, MS: middle shading（58% shading）, LS: low shading（34% shading）, TH: thinning, CT: control.

treatment especially increased stem dry weight. Although the treatments changed dry matter distribution between the leaves and the stems, they did not affect morphological characteristics such as leaf area index （LAI）, plant length and tiller number（data not shown）.

### 3．Effects of shading and thinning on NSC content in the stems

Shading significantly decreased stem NSC concentration in both cultivars（Table 2）. Stem NSC concentration in high-shaded Koshihikari and Nipponbare plants, in particular, was greatly decreased by 33% and 38%, respectively, compared to that in the control plants. The amount of NSC in the stems, which is the product of NSC concentration and dry weight, was decreased significantly by shading. Thinning significantly increased NSC concentration in both cultivars. Because of the increased stem dry weight, the amount of NSC in the stems of both cultivars was increased significantly by thinning.

### 4．Relationship between amount of available carbohydrate and final spikelet number

Hypothesis I states that final spikelet number is determined by the amount of available carbohydrate during the mid-reproductive stage. Available carbohydrate was defined as shoot dry matter production during the treatments or the amount of NSC in the stems at the end of the treatments. Both dry matter production and NSC amount had relatively high, positive correlations with final spikelet number within each cultivar（Table 3）. However, the regression lines were so different between both the cultivars that the regression coefficients between final spikelet number and two kinds of available carbohydrate, grouping both cultivars together, were not positive（Table 3）. Assimilation is strongly related to LAI and N content, but final spikelet number was not correlated with either LAI or shoot N content per hill at the end of the treatments（Table 3）.

**Table** 3.　Regression coefficients between the number of final spikelets and several parameters.

| | NSC amount | Dry matter production | LAI | Nitrogen amount |
|---|---|---|---|---|
| Koshihikari | 0.588 ns | 0.696 ns | 0.439 ns | -0.100 ns |
| Nipponbare | 0.802 ns | 0.567 ns | -0.161 ns | -0.893 * |
| Both cultivars | -0.068 ns | -0.563 ns | -0.778 * | -0.910 ** |

NSC amount is the amount of non-structural carbohydrate in stem at the end of the treatments（at the meiosis stage）.
Dry matter production is shoot dry matter production during the treatments（the mid-reproductive stage）.
LAI is leaf area index at the end of the treatments.
Nitrogen amount is the amount of shoot nitrogen at the end of the treatments.
* and ** show significant at 5% and 1% level of probability, respectively.
ns shows not significant at 5% level of probability.

### 5. Relationship between amount of available carbohydrate per differentiated spikelet and number of degenerated spikelets

Hypothesis II states spikelet degeneration percentage is determined by the amount of available carbohydrate per differentiated spikelet. Dry matter production per differentiated spikelet had a high, negative correlation with spikelet degeneration percentage (Fig. 2, r=−866**). The amount of NSC per differentiated spikelet at the end of the treatments also had a high, negative correlation with spikelet degeneration percentage (Fig. 3, r=−0.835**). Contrary to the results in Table 3, these two relations held between the two cultivars.

## Discussion

Spikelet degeneration percentage was highly, negatively correlated with both dry matter production per differentiated spikelet during the mid-reproductive stage and the amount of NSC per differentiated spikelet at the meiosis stage. This result supports Hypothesis II, that available carbohydrate per differentiated spikelet determines spikelet degeneration percentage and then determines final spikelet number. The hypothesis II is based on the assumption that differentiated spikelets compete with each other for assimilates during the mid-reproductive stage when spikelet growth is vigorous. Therefore, survival percentage of differentiated spikelets would depend on how much available carbohydrate can be supplied to each differentiated spikelet.

In contrast with Hypothesis II, final spikelet number has only a weak relation to dry matter production and stem NSC amount in the mid-reproductive stage. This means that final spikelet number is not proportional to the amount of available carbohydrate in rice (Hypothesis I). Fischer (1985) related kernel number per $m^2$ in spring wheat linearly with the photothermal quotient, which is the ratio of incident solar radiation to accumulated effective temperature in the critical preanthesis period based on the assumption that dry matter production and partitioning determine kernel number. Hasegawa et al. (1994) developed a model of spikelet number per unit area in rice using two parameters: N concentration and shoot dry weight. The asymptotic response of spikelet number to dry weight in the model was related to soluble carbohydrate content of the crop. This asymptotic response implies that a part of the dry matter would not be used for producing spikelets. This phenomenon can be compared with partial ripening in rice grains, to which wasteful carbohydrate is distributed. It is possible that a part of the NSC would be wastefully distributed to degenerating spikelets.

The mechanism of spikelet degeneration is little known. Most of the degenerated spikelets are on basal branches, and

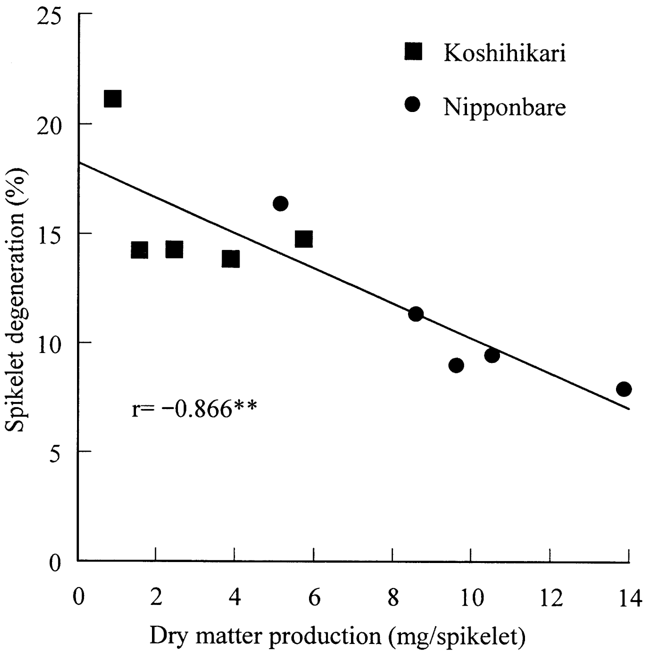

**Fig. 2.** The effects of the dry matter production in the shoots per differentiated spikelet during the shading and thinning treatments on the spikelet degeneration percentage.

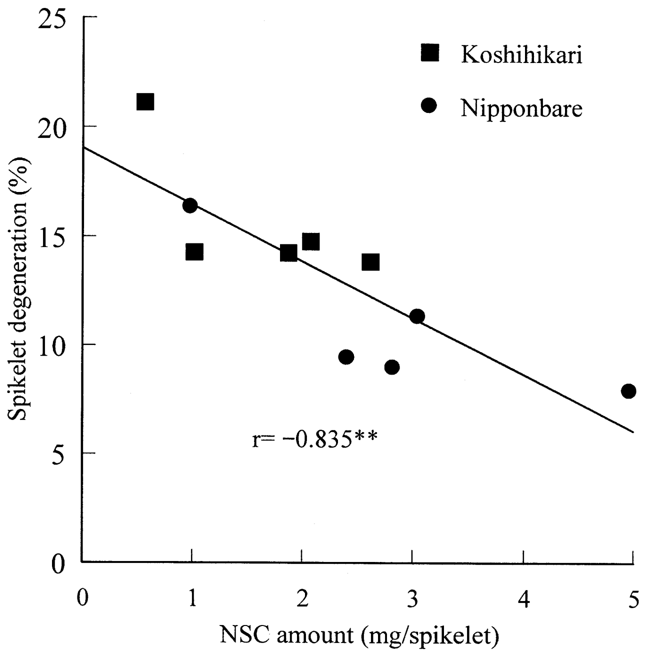

**Fig.3.** The effects of the amount of non-structural carbohydrate (NSC) in the stems per differentiated spikelet at the end of the shading and thinning treatments on the spikelet degeneration percentage.

spikelets on distal branches rarely degenerate（Patel and Mohapatra，1992；Kobayasi and Imaki，1997）．However, no clear causal relationship could be detected between assimilate concentration and spikelet development when comparing upper and lower primary rachis-branches （Mohapatra and Sahu，1991）．Indica rice, which has larger number of spikelets per panicle, has more large vascular bundles passing a neck node of a panicle per primary rachis-branch than japonica rice. These additional large vascular bundles of indica rice differentiate at the early spikelet differentiation stage（Fukushima and Akita，1997）．The degree of development in these large vascular bundles might control the supply of NSC with differentiated spikelets. In pod abortion of pigeon peas, Sheldrake（1979）developed a hydrodynamical model based on the notion of competition. In this model, pod abortion is determined by competition for available assimilates among pods. The tube diameter in his model, which limits the supply of carbohydrate, could be compared to the degree of development in large vascular bundles in rice panicles.

During the reproductive stage, panicle development competes with carbohydrate saving for ripening and internode elongation. From long-term experimental data of two cultivars, namely Manryo（japonica）and IR29723−3−2−1（indica），Akita（1991）derived a linear relationship between spikelet number and crop growth rate during the reproductive stage minus the amount of NSC at heading. From this relationship, he assumed that sink formation and NSC saving for ripening would compete and that higher accumulation of NSC may be competitive and conflicting to sink size formation. Elongation of culms, a large potentially competitive sink for assimilates, coincides approximately with panicle differentiation and development（Murata and Matsushima，1975；Pattanaik and Mohapatra，1988）．These facts show that the development of spikelets would compete for assimilates not only with each other but also with stem NSC accumulation and internode elongation. Advanced estimation of the partitioning of available carbohydrate between spikelets and other organs will be necessary for decreasing degenerated spikelets.

In this experiment, dry matter production during the mid-reproductive stage was not proportional to final spikelet number. This result suggests that differentiated spikelets will compete for available carbohydrate at the meiosis stage, and a part of carbohydrate might be used wastefully for degenerated spikelets. To increase spikelet number, it will be important to use available carbohydrate efficiently for spikelet production through optimizing the distribution of assimilates among spikelets and other organs. Advanced research is needed on the mechanism and the quantification in competition among spikelets as a final sink and other organs.

## References

Akita, S.: The relationship between sink size in rice and the amount of accumulated non-structural carbohydrate. Jpn. J. Crop Sci., 60（Extra 1）：88−89, 1991.*

Dayan, E. H., van Keulen, H. and Dovrat, A.: Tiller dynamics of rhodes grass after defoliation: A model called TILDYN. Agro-Ecosystem，7：101−112, 1981.

Fischer, R. A.: Number of kernels in wheat crops and the influence of solar radiation and temperature. J. Agric. Sci., Cambridge，105：447−461, 1985.

Fukushima, A. and Akita, S.: Varietal differences of the course and differentiation time of large vascular bundles in the rachis of rice. Jpn. J. Crop Sci., 66：24−28, 1997.**

Hasegawa, T., Koroda, Y., Seligman, N. G., and Horie, T.: Response of spikelet number to plant nitrogen concentration and dry weight in paddy rice. Agron. J., 86：673−676, 1994.

Kobayasi, K.: The analysis of the process in spikelet number determination with special reference to nitrogen nutrition in rice. Bull. Fac. Life Env. Sci. Shimane Univ., 5：13−17, 2000.

Kobayasi, K. and Imaki T.: Varietal differences of rice in differentiation and degeneration of secondary rachis-branches and spikelets in terms of their nodal distribution on a rachis. Jpn. J. Crop Sci., 66：578−587, 1997.**

Matsushima, S.: Analysis of developmental factors determining yield and yield prediction in low land rice. Bull. Natl. Inst. Agric. Sci., A5：1−271, 1957.***

Matsushima, S.: Theories and Techniques in Rice Cultivation −Theory on Yield Analysis and Its Application. Yokendo, Tokyo，1959.*

Mohapatra, P. K. and Sahu, S. K.: Heterogeneity of primary branch development and spikelet survival in rice panicle in relation to assimilate of primary branches. J. Exp. Bot., 42：871−879, 1991.

Murata, Y. and Matsushima, S: Rice. In Evans, L. T.,（ed.） Crop Physiology, Cambridge University Press, London, pp. 73−99, 1975.

Patel, R. and Mohapatra, P. K: Regulation of spikelet development in rice by hormones. J. Exp. Bot., 43：257－262, 1992.

Pattanaik, P. K. and Mohapatra, P. K.: Role of assimilates and phosphates in the control of internode elongation in tall and dwarf indica rice varieties. J. Exp. Bot., 39：1367－1380, 1988.

Senanayake, N., De Datta, S. K., Naylor, R. E. L., and Thompson, W. J.: Lowland rice apical development: Stages and cultivar differences detected by electron microscopy. Agron. J., 83：1013－1023, 1991.

Senanayake, N., Naylor, R. E. L. and De Datta, S. K.: Effect of nitrogen fertilization on rice spikelet differentiation and survival. J. Agric. Sci., Cambridge, 127：303－309, 1996.

Sheldrake, A. R.: A hydrodynamical model of pod-set in pigeonpea （*Cajanus cajan*）. Indian J. Plant Physiol., 22：136－143, 1979.

Wada, G.: The effect of nitrogenous nutrition on the yield－Determining process of rice plant. Bull. Natl. Inst. Agric. Sci., A16：27－167, 1969. ***

*: In Japanese.

**: In Japanese with English abstract.

***: In Japanese with English summary.