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Correlation of Cytokinin Levels in the Endosperms and Roots with Cell Number and Cell Division Activity during Endosperm Development in Rice

JIANCHANG YANG[1], JIANHUA ZHANG[2,*], ZULIU HUANG[1], ZHIQING WANG[1], QINGSEN ZHU[1] and LIJUN LIU[1]

[1]*College of Agriculture, Yangzhou University, Yangzhou, Jiangsu, China and* [2]*Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China*

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Cell number and cell division activity in rice (*Oryza sativa*) endosperms are possibly regulated by cytokinin levels in the endosperm and its source in the roots. This study tried to find the possible correlations among them. Six rice genotypes were grown in nutrient solution. Two patterns of endosperm cell division, synchronous and asynchronous, were observed among the genotypes based on the cell division rate of superior and inferior spikelets. Contents of zeatin (Z) + zeatin riboside (ZR) were much higher than those of N^6-isopentenyladenine (iP) and N^6-isopentenyladenosine (iPR) in both endosperms and roots. Changes in Z + ZR levels in endosperms were significantly correlated with those in roots, and both were very significantly correlated with the cell division rate. Changes in iP + iPR contents in the roots were not significantly correlated with those in the endosperms and the cell division rate. When roots were treated with kinetin, endosperm cell number and grain weight were increased. Such enhancement was more significantly achieved by the root kinetin treatment than by spraying kinetin on leaves and panicles. The results suggest that the cell number and cell division activity in rice endosperms are regulated by cytokinin levels in the endosperm and that root-derived Z + ZR play a pivotal role.

Key words: Cell division activity, cytokinin, endosperm, rice, *Oryza sativa* L., root.

INTRODUCTION

The endosperm of rice (*Oryza sativa* L.) contributes more than 90 % of the final weight of a caryopsis (Murata and Matsushima, 1975; Cao *et al*., 1992). The process of grain filling is actually the increase in cell number and cell filling in the endosperm. There is generally a positive relationship between endosperm cell number and grain weight in wheat (*Triticum aestivum* L.; Gleadow *et al*., 1982; Gao *et al*., 1992), barley (*Hordeum vulgare* L.; Cochrane and Duffus, 1981, 1983), maize (*Zea mays*; Jones *et al*., 1985) and rice (Yang *et al*., 2000*a*). Thus, endosperm cell number is one important factor determining grain weight.

Since the endosperm cell number in cereal crops is established during the early phases of grain development, it is speculated that this parameter may be regulated by cytokinins rather than by the levels of available carbohydrates during grain filling (Jones *et al*., 1992; Brenner and Cheikh, 1995). There are many reports that the highest concentrations of cytokinins are generally found in the developing seeds of peas (*Pisum* sp.), beans (*Phaseolus* sp.), maize, wheat and rice, which coincides with the period of seed set and rapid endosperm development (Jones *et al*., 1992; Lur and Setter, 1993; Morris *et al*., 1993, Yang *et al*., 2000*b*). Laureys *et al*. (1998) observed that zeatin-type cytokinins played a specific role in tobacco (*Nicotiana* sp.) cell division. Although cytokinins are generally considered to play a major role in promoting cell division, no information is available with regard to their effect on endosperm cell number and it is not known whether cytokinin levels in the endosperm are correlated with endosperm cell division activity.

Based on their flowering date and locations within a panicle, rice spikelets can be classified as superior or inferior (Zhu *et al*., 1988; Iwasaki *et al*., 1992). In general, superior spikelets flower earlier and are located at the top of primary branches, whereas inferior spikelets flower later and are located at the base of secondary branches. Superior spikelets usually produce heavier grains than inferior ones (Sikder and Gupta, 1976; Kato, 1989; Yang *et al*., 2000*b*). It is of interest to know whether this difference is related to cytokinin levels in the endosperm.

The objectives of this study were to investigate changes in cytokinin level in the endosperms and roots, and to determine whether the hormone is correlated with cell number and cell division activity during rice endosperm development.

MATERIALS AND METHODS

Plant materials

The experiment was conducted at Yangzhou University farm, Jiangsu Province, China (32°30′N, 119°25′E) during the rice growing season (May to October) of 1999, and repeated in 2000. Six rice (*Oryza sativa* L.) genotypes, Yanjin 2 and Sujing 1 (japonica inbred cultivars), IR72 and Yangdao 4 (indica inbred cultivars), and JW-8/IR36 and PC/

* For correspondence. Fax +852 2339 5995, e-mail jzhang@hkbu.edu.hk

Zao (japonica/indica F_1 hybrids), were grown using the water culture method described by Mae and Ohira (1981). On 30 May, 20-d-old seedlings that had been raised in a paddy field were transplanted into nine cement tanks with a hill spacing of 0·20 m × 0·15 m with one seeding per hill. Each tank was 8 m long, 2 m wide, 0·4 m high and had a volume of 6·4 m^3. The tanks were built in a farm field. The basal nutrient solution (full strength) consisted of: 1·4 mM NH_4NO_3, 0·4 mM NaH_2PO_4, 0·5 mM K_2SO_4, 1·5 mM $MgSO_4$, 1 mM $CaCl_2$, 1·6 mM $MnCl_2$, 0·2 mM $(NH_4)_6Mo_7O_{24}$, 3 mM H_3BO_3, 6 mM $FeCl_3$, 0·03 mM $ZnSO_4$ and 0·03 mM $CuSO_4$. The solution was changed once a week and its pH was adjusted every day to 5·0 with 1 N HCl or 1 N NaOH. Full strength nutrient solution was used from transplanting to heading, and three-quarter strength was used thereafter. All the genotypes (50 % of plants) headed between 20–23 August, and were harvested between 6–8 October. The temperatures, averaged every 10 d from anthesis (21–23 August) to harvest, were 26·9, 26·3, 25·2, 24·3, 23·2 and 22·6 °C, respectively.

Sampling

Six hundred panicles that headed on the same day were chosen and tagged for each genotype. The flowering date and the position of each spikelet on the tagged panicles were recorded. At 2 d intervals from anthesis to 28 days post anthesis (DPA), 30–36 tagged panicles of each genotype, along with their stems and roots, were sampled. The sampled panicles (including roots) were divided into three sub-samples of 10–12 panicles each. Superior spikelets that flowered on the first 2 d within a panicle and inferior ones that flowered on the last 2 d within a panicle were separated from the sampled panicles. The difference in flowering date between superior and inferior spikelets was 3–5 d within a panicle. Roots from 10–12 stems and 100–120 grains from superior or inferior spikelets formed each sample. These were frozen in liquid nitrogen for 1 min and then stored at –80 °C prior to hormonal assay. After cutting a small hole in the edge of the hull, 10–12 grains from superior or inferior spikelets were fixed in Carnoy's solution (absolute ethanol: glacial acetic acid : chloroform, 9 : 3 : 1, v/v) for 48 h, then kept in 70 % (v/v) ethanol pending examination of endosperm cell number. Twenty plants (165–184 stems) from each genotype were harvested at physiological maturity for measurement of endosperm and grain weight.

Nuclear/cell counting

The method for isolation and counting of endosperm cells was modified from Singh and Jenner (1982). Briefly, fixed grains were dehulled and transferred to 50 % (v/v) then 25 % (v/v) ethanol and finally into distilled water for 5 to 7 h prior to dissection of the endosperm. The endosperm was isolated under a dissecting microscope, stained with Delafied's haematoxylin solution for 24–30 h, washed several times with distilled water and then hydrolysed in 0·1 % (w/v) cellulase (No. c-2415; Sigma Chemical Co., St Louis, MO, USA) solution (pH 5·0) at 40 °C for 4–6 h and oscillated. Isolated endosperm cells were diluted to 2–10 ml according to the developmental stage of the endosperm, from which eight to ten sub-samples (20 µl for each sub-sample) were transferred to a counting chamber (1 cm^2 area). Using a light microscope, the endosperm cell number of ten fields of view for each counting chamber was noted. Within 2 DPA for superior spikelets and 4–6 DPA for inferior ones, the number of nuclei was taken as endosperm cell number. The total cell number per endosperm was calculated according to Liang *et al*. (2001). Six grains (endosperms) per genotype were examined at each measurement.

The division process of endosperm cells was fitted by Richards' (1959) growth equation as described by Zhu *et al*. (1988):

$$M = A/(1 + Be^{-kt})^{1/N} \quad (1)$$

where M is cell number, A the maximum cell number, t is time after anthesis (d), and B, k, and N are coefficients determined by regression.

The rate of endosperm cell division (R) was calculated as the derivative of eqn (1):

$$R = AKBe^{-kt}/N(1 + Be^{-kt})^{(N+1)/N} \quad (2)$$

The active period of endosperm cell division was defined as that when M was from 5 (t_1) to 95 % (t_2) of A. The average rate of endosperm cell division during this period was calculated from t_1 to t_2.

Cytokinin extraction, purification and quantification

The methods for extraction and purification of zeatin (Z), zeatin riboside (ZR), N^6-isopentenyladenine (iP) and N^6-isopentenyladenosine (iPR) were modified from those described by Bollmark *et al*. (1988) and He (1993). Samples consisting of 50–80 dehulled, frozen grains with their embryos removed, or 3–4 g frozen roots, were ground in an ice-cooled mortar in 10 ml 80 % (v/v) methanol extraction medium containing 1 mM butylated hydroxytoluence (BHT) as an antioxidant. The extract was incubated at 4 °C for 4 h and centrifuged at 4000 rpm for 15 min at the same temperature. The supernatant was passed through Chromosep C18 columns (C18 Sep-Park Cartridge; Waters Corp., Millford, MA, USA), pre-washed with 10 ml 100 % and 5 ml 80 % methanol. The hormone fractions eluted from the columns using 10 ml 100 % methanol and 10 ml ether were dried under N_2, and dissolved in 2 ml phosphate-buffered saline (PBS) containing 0·1 % (v/v) Tween 20 and 0·1 % (w/v) gelatine (pH 7·5) for analysis by enzyme-linked immunosorbent assay (ELISA).

The mouse monoclonal antigens and antibodies against Z, ZR, iP and iPR, and immunoglobulin G-horse radish peroxidase (Ig G-HRP) used in ELISA were produced at the Phytohormones Research Institute, China Agricultural University, China (see He, 1993). The method for quantification of Z, ZR, iP and iPR by ELISA has been described previously (Yang *et al*. 2001). Each measurement was replicated six times.

Cross-activities for the antibodies used were very small (Table 1). The specificities of the monoclonal antibodies

TABLE 1. *Cross-activities (%) of the antibodies used against zeatin (Z), zeatin riboside (ZR), N^6-isopentenyladenine (iP) and N^6-isopentenyladenosine (iPR)*

Antibodies against:	Z	ZR	iP	iPR
Z	100	<0·1	<0·05	<0·05
ZR	<0·1	100	<0·05	<0·05
iP	<0·05	<0·05	100	<0·1
iPR	<0·05	<0·05	<0·1	100

and the possibility other non-specific immunoreactive interference have been checked by Wu *et al*. (1988), Zhang *et al*. (1991) and He (1993); the method proved reliable.

Exogenous kinetin application

Plants of two genotypes, Yangdao 4 and PC/Zao, were grown using the liquid culture method described above. Kinetin (6-furfurylaminopurine; Sigma Chemical Co.) was dissolved in 0·1 N NaOH (pH 7·1). Two treatments were conducted. In treatment one (T1), dissolved kinetin was added to the culture solution at a concentration at 10^{-7} M for 4 d starting at anthesis, and leaves and panicles was sprayed with 0·5 % (v/v) Teepol (Fluka, Riedel-de-Haen, Germany) solution. In treatment two (T2), dissolved kinetin was diluted with distilled water to a concentration at 50×10^{-6} M and then sprayed onto leaves and panicles daily for 4 consecutive days with 0·5 % Teepol as surfactant. Plants sprayed with the same volume of 0·5 % Teepol solution were used as a control. Each treatment was performed on 200 plants (1650–1780 stems). The number of endosperm cells was examined at 4 d intervals from 6–28 DPA so that the maximum cell number could be observed. Twenty plants per treatment were harvested at physiological maturity for examination of endosperm and grain weight.

Statistical analysis

Results were analysed using the SAS statistical analysis package (version 6·12; SAS Institute, Cary, NC, USA). Data from each sampling date were analysed separately. Means were tested using the least significant difference at $P_{0.05}$ ($LSD_{0.05}$). Linear regression was used to evaluate the relationship between cytokinin contents in roots and endosperms and the rate of endosperm cell division.

RESULTS

Cell division rate and cell number

Based on the difference between superior and inferior spikelets in terms of their endosperm cell division rates, the six genotypes tested could be classified into two categories showing either a synchronous or an asynchronous pattern (Fig. 1). In the synchronous pattern (genotypes Yanjing 2, Sujing 1 and IR72), division of endosperm cells in both superior and inferior spikelets was rapid immediately after

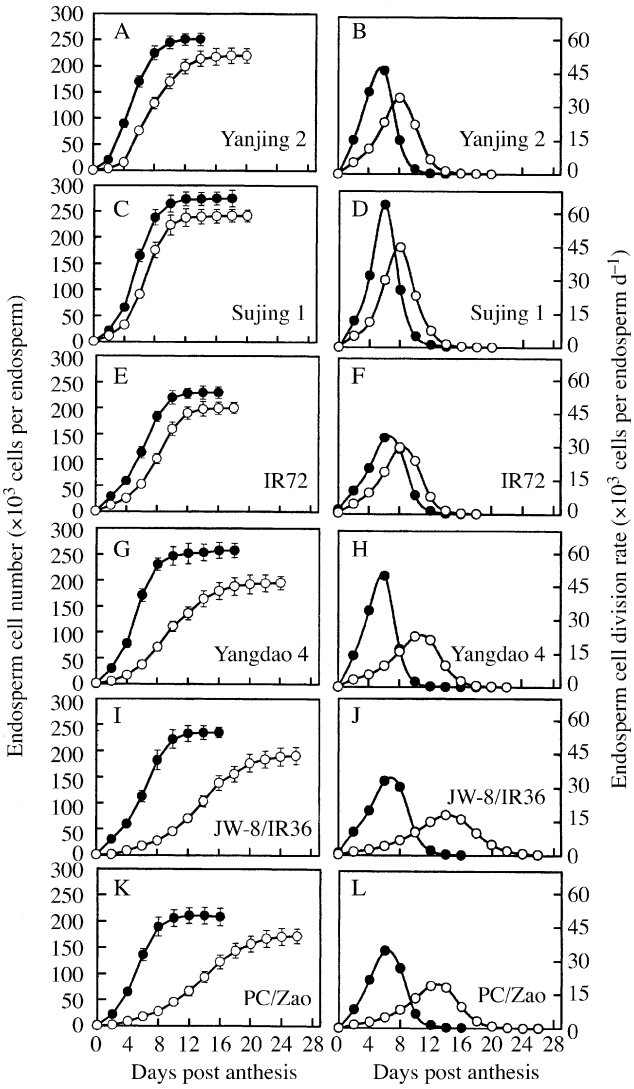

FIG. 1. Cell numbers and cell division rates in the endosperms of superior (closed circles) and inferior (open circles) spikelets of rice. The genotypes studied are japonica cultivars Yanjing 2 (A and B) and Sujing 1 (C and D), indica cultivars IR72 (E and F) and Yangdao 4 (G and H), and japonica/indica hybrids JW-8/IR36 (I and J) and PC/Zao (K and L). The division rate of endosperm cells was calculated according to Richards' (1959) equation. Vertical bars in A, C, E, G, I and K represent ± s.e.m. (n = 6) where these exceed the size of the symbol.

they were fertilized. The maximum cell division rate and the maximum cell number were attained at 6 and 12 DPA, respectively, for superior spikelets, and at 8 and 14–16 DPA, respectively, for inferior ones. In the asynchronous pattern (genotypes Yangdao 4, JW-8/IR36 and PC/Zao), endosperm cell division in superior spikelets was much faster during early endosperm development and the maximum division rate was attained much earlier than that in inferior spikelets. The maximum division rate and the maximum cell number occurred at 6 and 12–14 DPA, respectively, for superior spikelets, and at 12–14 and 22–24 DPA, respectively, for inferior spikelets.

Compared with their respective inferior spikelets, superior spikelets of the six genotypes had a shorter period

TABLE 2. *The rate and active period of endosperm cell division, maximum endosperm cell number, cell weight, endosperm weight and grain weight of rice*

Genotype	Type of spikelet	Mean cell division rate ($\times 10^3$ cells per endosperm d^{-1})	Active division period (DPA)	Endosperm weight (mg)	Maximum cell number ($\times 10^3$ cells per endosperm)	Cell weight (ng)†	Grain weight (mg)
Yanjing 2	Superior	25·2^{bc}	2–10	23·3^{bc}	252^{bc}	92·4^{a}	29·0^{bc}
	Inferior	18·0^{e}	4–14	20·9^{e}	220^{ef}	95·0^{a}	26·1^{ef}
Sujing 1	Superior	27·5^{a}	2–10	24·8^{a}	275^{a}	90·2^{a}	30·8^{a}
	Inferior	24·2^{c}	4–12	22·7^{cd}	242^{cd}	93·8^{a}	28·2^{bc}
IR72	Superior	25·9^{b}	2–9	21·9^{d}	231de	94·8^{a}	26·8de
	Inferior	18·0^{e}	3–12	18·8^{fg}	200^{gh}	94·0^{a}	24·5^{gh}
Yangdao 4	Superior	25·7^{b}	2–10	23·8^{b}	257^{b}	92·6^{a}	29·2^{b}
	Inferior	13·5^{f}	6–18	18·4^{gh}	195^{h}	94·4^{a}	24·7^{g}
JW-8/IR36	Superior	21·2^{d}	2–11	22·1^{d}	236^{d}	93·6^{a}	27·8^{cd}
	Inferior	11·5^{g}	6–20	17·6^{h}	191^{h}	92·2^{a}	23·3^{h}
PC/Zao	Superior	21·2^{d}	2–10	19·5^{f}	212^{fg}	92·0^{a}	25·4^{fg}
	Inferior	11·8^{g}	6–18	13·7^{i}	171^{i}	80·1^{b}	18·1^{i}

The mean cell division rate and active cell division period were calculated according to Richards' (1959) equation. Values of endosperm weight and grain weight are means of 20 plants harvested from each genotype.

Means followed by different superscripts indicate significant differences at $P = 0{\cdot}05$.

† Endosperm weight/ maximum endosperm cell number.

TABLE 3. *Recovery test of ELISA for zeatin (Z), zeatin riboside (ZR),* N^6*-isopentenyladenine (iP) and* N^6*-isopentenyladenosine (iPR)*

Plant organ	Hormone	Plant sample (pmol g fresh wt^{-1})†	Plant sample + standard (pmol g fresh wt^{-1})‡	Recovery (%)
Endosperm	Z	501 ± 48·1	901 ± 91·3	79·8 ± 7·2
	ZR	724 ± 70·2	1133 ± 115	81·7 ± 8·8
	iP	120 ± 20·9	508 ± 55·7	77·6 ± 8·1
	iPR	145 ± 9·2	521 ± 51·3	75·3 ± 7·7
Roots	Z	581 ± 64·5	964 ± 98	76·6 ± 7·9
	ZR	907 ± 88·5	1298 ± 124	78·1 ± 7·4
	iP	122 ± 12·8	489 ± 50·3	73·4 ± 7·6
	iPR	163 ± 16·4	553 ± 57·1	77·9 ± 7·9

The test cultivar was Yangdao 4 (indica). Data are expressed as means ± s.e. of six replications.

Five hundred picomole of each compound was added to 1 g fresh plant sample before purification. The compounds were from Sigma Chemical Co.

† Plant sample only.

‡ Plant sample + synthetic compounds of Z, ZR, iP, and iPR, respectively.

of active division, a faster rate of division and a greater number of endosperm cells (Fig. 1; Table 2). The periods of active division of endosperm cells lasted 8–10 d for superior spikelets and 9–15 d for inferior ones (Table 2). Mean division rates during the active cell division period were $21{\cdot}2 \times 10^3$ to $27{\cdot}5 \times 10^3$ cells per endosperm d^{-1} for superior spikelets, and $11{\cdot}5 \times 10^3$ to $24{\cdot}2 \times 10^3$ cells per endosperm d^{-1} for inferior ones. The maximum number of endosperm cells in superior spikelets was 212×10^3 to 275×10^3 per endosperm, compared with 171×10^3 to 242×10^3 cells per endosperm in inferior spikelets. Differences between superior and inferior spikelets in the division rate and the maximum number of endosperm cells were greater in genotypes showing asynchronous cell division than in those exhibiting synchronous cell division. Differences in weight per endosperm cell were not significant between superior and inferior spikelets or among genotypes, with the exception of inferior spikelets in PC/Zao (Table 2). Generally, a spikelet or genotype with a higher mean division rate of endosperm cells also had more endosperm cells, and a greater endosperm weight and grain weight (Table 2). Regression analysis showed that all these parameters were positively and significantly correlated ($r = 0{\cdot}86$–$0{\cdot}98$, $P < 0{\cdot}01$).

Changes in cytokinin levels in endosperms

The methods used in this study for cytokinin extraction and purification and for quantification of cytokinins using ELISA recovered 78·9 % of Z, 78·1 % of ZR, 74·4 % of iP and 77·8 % of iPR. The recovery was similar between endosperms and roots (Table 3).

Endosperms of superior and inferior spikelets contained 38–44 % more ZR than Z and 17–21 % more iPR than iP. Z and ZR or iP and iPR showed a similar pattern of change during endosperm development (data not shown). In

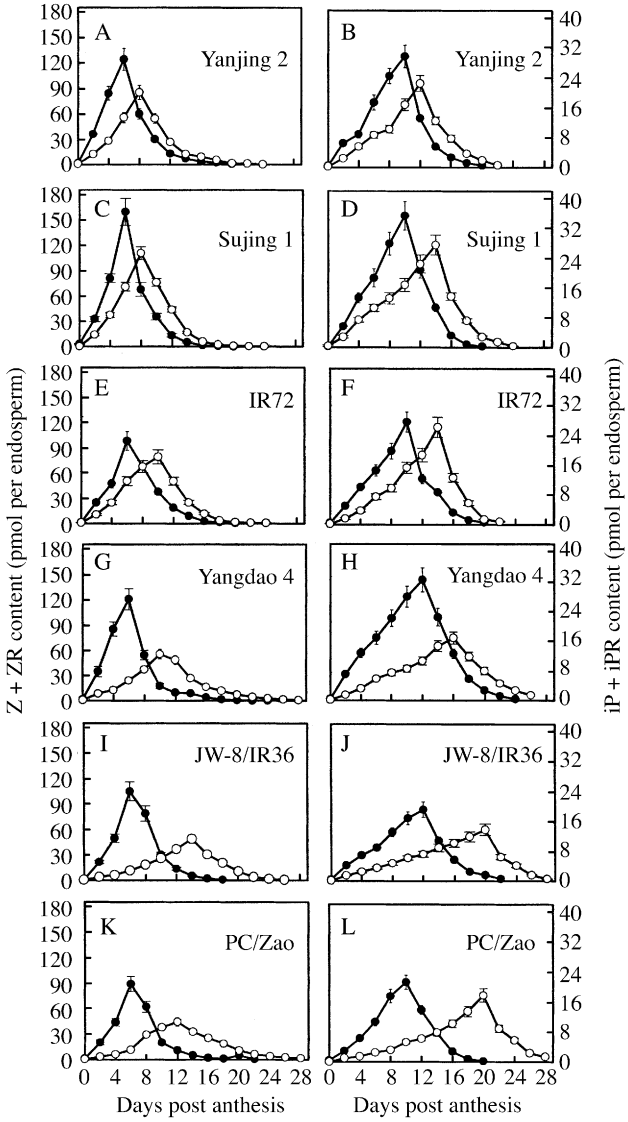

FIG. 2. Changes of Z + ZR and iP + iPR contents in the endosperms of superior (closed circles) and inferior (open circles) spikelets of rice. The test genotypes are japonica cultivars Yanjing 2 (A and B) and Sujing 1 (C and D), indica cultivars IR72 (E and F) and Yangdao 4 (G and H), and japonica/indica hybrids JW-8/IR36 (I and J) and PC/Zao (K and L). Vertical bars represent ± s.e.m. (n = 6) where these exceed the size of the symbol.

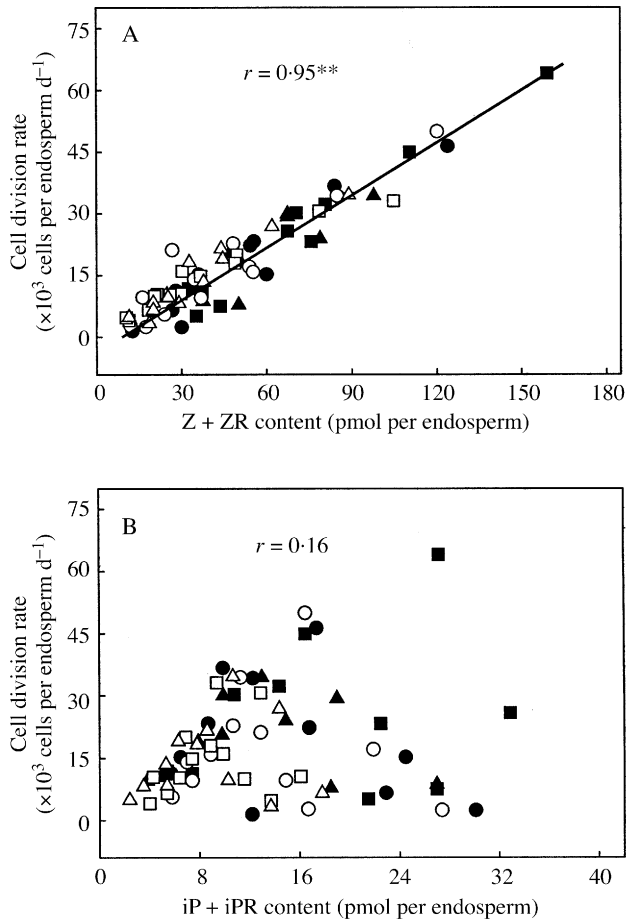

FIG. 3. Relationship between rice endosperm cell division rate and the content of Z + ZR (A) and iP + iPR (B) in endosperms during the period of active cell division. The test genotypes are japonica cultivars Yanjing 2 (closed circles) and Sujing 1 (closed squares), indica cultivars IR72 (closed triangles) and Yangdao 4 (open circles), and japonica/indica hybrids JW-8/IR36 (open squares) and PC/Zao (open triangles). Data are from Figs 1 and 2. Correlation coefficients (r) are calculated and asterisks represent statistical significance at P = 0·01.

genotypes with a synchronous pattern of cell division, Z + ZR contents increased transiently in the endosperms of both superior and inferior spikelets during early endosperm development. They reached a maximum at 6–8 DPA, and declined sharply thereafter (Fig. 2A, C, E, G, I and K). For genotypes with an asynchronous pattern of cell division, Z + ZR contents in the endosperm of inferior spikelets increased slowly during early endosperm development, reached a maximum at 12–14 DPA and declined slowly thereafter. Superior spikelets of genotypes exhibiting asynchronous cell division had a much higher Z + ZR content in the endosperm than inferior spikelets.

Changes in iP + iPR contents in endosperms were similar to those of Z + ZR (Fig. 2B, D, F, H, J and L). However, iP + iPR contents were much lower than those of Z + ZR, at only about 25 % of the latter. Maximum iP + iPR content was achieved later than that for Z + ZR: peak values occurred at 10–14 DPA for superior spikelets and 14–20 DPA for inferior ones, towards the end of endosperm cell division.

Z + ZR contents in the endosperm paralleled the rate of endosperm cell division during the active division period (see Fig. 1). Regression analysis demonstrated that Z + ZR content was positively and significantly correlated with the rate of cell division (r = 0·95, P < 0·01; Fig. 3A). When all data were considered, the correlation between iP + iPR content and the rate of cell division was not significant (r = 0·16, P > 0·05; Fig. 3B). However, for low iP + iPR amounts, e.g. up to 12 pmol per endosperm, the cell division rate and iP + iPR content were positively and significantly correlated (r = 0·88, P < 0·01; Fig. 3B). Mean values of iP + iPR contents during the active cell division period were also

TABLE 4. *Correlations between mean values of Z + ZR and iP + iPR in endosperms during the active period of endosperm cell division and the mean division rate and the maximum number of endosperm cells in rice*

Correlation between	Mean cell division rate	Maximum cell number
Mean values of Z + ZR	0·93**	0·88**
Mean values of iP + iPR	0·87**	0·84**

Data used in the calculation are from Table 1 and Fig. 2.
** correlation significance at $P = 0{\cdot}01$ ($n = 12$).

positively correlated with both the mean rate of division of endosperm cells ($r = 0{\cdot}87$, $P < 0{\cdot}01$) and maximum cell number ($r = 0{\cdot}84$, $P < 0{\cdot}01$) (Table 4).

Changes in cytokinin levels in roots

Similar to those in the endosperms, Z + ZR contents in the roots were also related to the rate of endosperm cell division (Fig. 4A). Genotypes with a faster rate of endosperm cell division also had more Z + ZR in the roots during early endosperm development. Root Z + ZR contents of genotypes exhibiting synchronous cell division showed some increase after anthesis, peaked at 4–6 DPA, then declined gradually. Genotypes showing asynchronous cell division exhibited two weak peaks of root Z + ZR content. The first peak occurred at 4–6 DPA and the second at 12–14 DPA, which coincided with the maximum rate of endosperm cell division in these genotypes.

In contrast to root Z + ZR contents, root iP + iPR contents were very low, and only about 12–14 % of Z + ZR contents (Fig. 4B). The content of iP + iPR in roots changed little during the first 10 DPA, and gradually decreased thereafter. Genotypes with a faster rate of cell division also showed a greater iP + iPR content in roots during early endosperm development.

Changes in Z + ZR contents in roots were positively and significantly correlated with Z + ZR contents in endosperms ($r = 0{\cdot}89$, $P < 0{\cdot}01$; Table 5) and with the rate of division of endosperm cells ($r = 0{\cdot}82$, $P < 0{\cdot}01$). Correlations of iP + iPR contents in roots with iP + iPR contents in endosperms and the cell division rate were not significant ($r = 0{\cdot}33$–$0{\cdot}36$, $P > 0{\cdot}05$; Table 5).

Effects of kinetin application

When kinetin was applied to plants during early endosperm development, both endosperm cell number and endosperm/grain weight increased (Table 6). This increase was more substantial in inferior than in superior spikelets, and was more pronounced when kinetin was applied to roots (T1) than when it was sprayed onto leaves and panicles (T2).

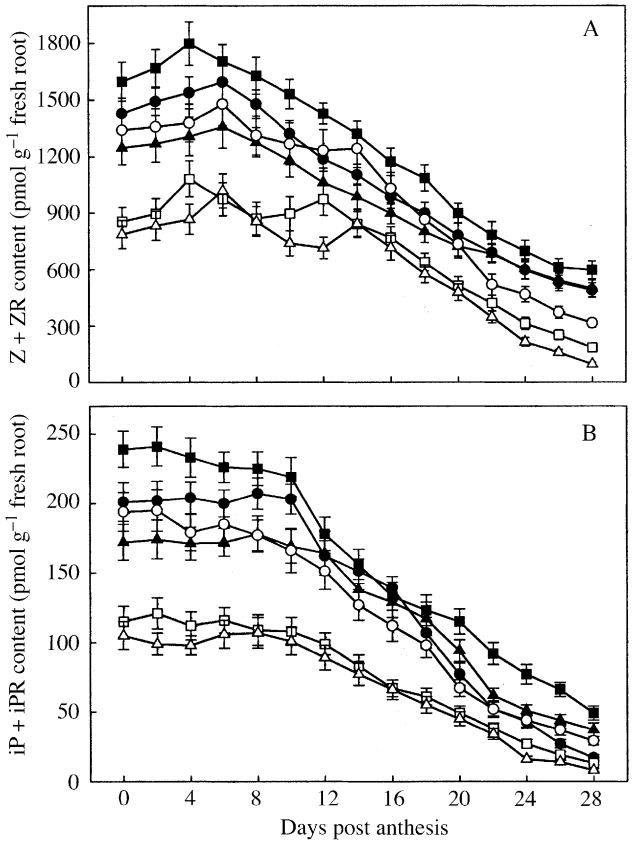

FIG. 4. Changes in Z + ZR (A) and iP + iPR (B) contents in the roots of rice. The test genotypes are japonica cultivars Yanjing 2 (closed circles) and Sujing 1 (closed squares), indica cultivars IR72 (closed triangles) and Yangdao 4 (open circles), and japonica/indica hybrids JW-8/IR36 (open squares) and PC/Zao (open triangles). Vertical bars represent ± s.e.m. ($n = 6$) where these exceed the size of the symbol.

TABLE 5. *Correlations between contents of Z + ZR in roots and endosperms and endosperm cell division rate, and correlations between contents of iP + iPR in roots and endosperms and endosperm cell division rate during the active cell division period in rice*

Correlation between	Z + ZR in endosperms	iP + iPR in endosperms	Endosperm cell division rate
Z + ZR in roots	0·89**	—	0·82**
iP + iPR in roots	—	0·36	0·33

Data used in the calculation are from Figs 1B (the mean cell division rate of superior and inferior spikelets), 2 (the mean Z +ZR and iP + iPR contents of superior and inferior spikelets) and 4.
** correlation significance at $P = 0{\cdot}01$ ($n = 42$).

DISCUSSION

Prior to this study, little information was available describing the endosperm cell division process in rice. Our results showed that the duration and rate of cell division in rice endosperms varied among genotypes and with the position of spikelets within a panicle (Fig. 1). Cell division in the endosperms ceased between 12 and 26 DPA, which contrasts with reports that endosperm cell division ceases at 8–16 DPA in wheat (Gleadow *et al.*, 1982; Gao *et al.*, 1992) and 28–30 DPA in barley (Cochrane and Duffus,

TABLE 6. *Effect of kinetin application on the maximum endosperm cell number and endosperm and grain weight of rice*

Genotype	Type of spikelet	Treatment	Maximum cell number ($\times 10^3$ cells endosperm^{-1})	Endosperm weight (mg)	Cell weight (ng)†	Grain weight (mg)
Yangdao 4	Superior	Control	259	23·9	92·3	29·1
		T1	268	24·7	92·2	29·9
		T2	264	24·4	92·4	29·6
	Inferior	Control	198	18·5	93·4	24·3
		T1	234**	22·3**	95·3	28·8**
		T2	227*	20·9*	92·1	26·5*
PC/Zao	Superior	Control	215	19·8	92·1	25·7
		T1	242*	22·5*	92·8	28·3*
		T2	231*	21·7*	93·9	27·6*
	Inferior	Control	173	14·1	81·5	19·1
		T1	204**	18·9**	92·6*	25·4**
		T2	185*	17·0**	91·7*	23·2**

Plants were grown in nutrient solution. In treatment T1, kinetin was added to the water culture solution at a concentration of 10^{-7} M for 4 d starting at anthesis. In T2, leaves and panicles were sprayed with 50×10^{-6} M kinetin daily for 4 d starting at anthesis. Measurement of endosperm cell number was replicated five times. Values of endosperm and grain weight were means of 20 plants harvested per genotype. Statistical comparison was within the same column, the same plant organ and the same genotype.

* $P = 0{\cdot}05$; ** $P = 0{\cdot}01$.

† Endosperm weight/maximum endosperm cell number.

1981). Thus, variation in the duration of endosperm cell division in rice is greater than that for either wheat or barley. We attribute such variation mainly to the heterogeneity of spikelet development.

Based on the rate of cell division of superior and inferior spikelets, two patterns of endosperm cell division, synchronous and asynchronous, were observed among the genotypes studied (Fig. 1). Cell number and cell division activity in the endosperms were closely associated with the pattern of cell division. Genotypes showing synchronous endosperm cell division had a faster rate of division, more endosperm cells and heavier endosperms/grains for both superior and inferior spikelets, compared with genotypes showing the asynchronous pattern (Table 2). These data suggest that grain weight might be improved by selecting progenies with the synchronous cell division pattern for breeding programmes.

Endosperm cell number is a function of the rate and duration of endosperm cell division. An increase in either the rate or the duration of division would increase cell number. However, we observed that the cell division duration seemed to be contrary to the cell division rate (Fig. 1). Superior spikelets or genotypes with a shorter cell division period generally had a higher rate of cell division (Table 2). The cell division rate, not duration, was positively and significantly correlated with the maximum cell number, and it was the cell number, not the weight per cell, that was significantly and positively correlated with the endosperm/grain weight. The results indicate that the endosperm cell division rate determines the endosperm cell number and grain weight in rice.

Inferior spikelets, especially those of genotypes showing asynchronous cell division, had a slow rate of cell division and a small number of cells (Table 2). Slow grain filling and low grain weight of inferior spikelets are generally attributed to constraints in assimilate availability (Venkateswarlu and Vesperas, 1987; Cao *et al*., 1992; Yuan, 1997). However, Mohapatra and co-workers (1991, 1993) reported that there was little difference in soluble carbohydrate, phosphate and free amino acid concentrations between basal and distal primary branches, or between late-flowering and early-flowering spikelets within a panicle during grain filling in rice. Using the same genotypes in this study, we also observed that sucrose content in inferior spikelets was double that in superior ones during early endosperm development (Wang *et al*., 1998; Yang *et al*., 1999). Thus, it can be concluded that the intrinsic concentration of assimilates should not limit the division rate of endosperm cells in inferior spikelets.

Radley (1978) suggested that the rate of cell division in wheat endosperms is regulated by growth factors other than sucrose concentration. Our data clearly showed that the rate of endosperm cell division was closely associated with cytokinin levels in endosperms (Fig. 2). During early endosperm development, inferior spikelets, especially those of genotypes exhibiting asynchronous cell division, had much lower cytokinin contents in their endosperms than superior spikelets. Changes in Z + ZR levels in endosperms paralleled and were significantly correlated with the rate of cell division (Fig. 3A). Although the correlation between iP + iPR contents and the rate of cell division was not significant when all data were considered (Fig. 3B), mean values of iP + iPR contents during the period of active cell division were positively correlated with the mean cell division rate and with the maximum cell number in the endosperm (Table 4). When kinetin was exogenously applied to either roots or panicles and leaves, the number of endosperm cells and endosperm/grain weight were significantly increased (Table 6). The results suggest that cytokinin levels in the endosperm may regulate the rate of endosperm cell division. Low cytokinin contents result in reduced cell division activity in the endosperm, leading to a

smaller endosperm cell number and low grain weight for inferior spikelets.

Changes in Z + ZR contents in the roots coincided with those in the endosperms (Fig. 4), and were positively and significantly correlated with Z + ZR contents in endosperms and with the rate of endosperm cell division (Table 5). The results support the proposal that roots are the major source of cytokinins for the grains (Carlson *et al*., 1987; Bano *et al*., 1993). Cytokinins are transported through the xylem into the shoots where physiology is regulated (Nooden *et al*., 1990; Kamboj *et al*., 1998). However, we observed that the changing profiles of iP + iPR contents in endosperms did not correspond to those in roots (Figs 2 and 4). The contents of iP + iPR in roots changed little during the first 10 DPA, whereas they increased greatly in endosperms during this period. Changes in iP + iPR contents in roots were not significantly correlated with those in endosperms (Table 5). These data imply that iP and iPR may not only be synthesized in roots, but also *de novo* biosynthesized in shoots, probably in the leaves and grains.

It is noteworthy that Z + ZR contents were much higher than iP + iPR contents in both roots and endosperms (Figs 2 and 4). The rate of endosperm cell division was significantly and positively correlated with the changes in Z + ZR contents in roots and endosperms, but not significantly correlated with those in iP + iPR (Fig. 3; Table 5). Endosperm cell number and endosperm weight were increased more by the root kinetin treatment than by spaying kinetin onto leaves and panicles (Table 6). The results suggest that root-derived Z + ZR play a pivotal role in the regulation of cell division in rice endosperms.

CONCLUSIONS

Our results demonstrate that cell number and cell division rate in rice endosperms vary among genotypes and with position of spikelets within a panicle. The variations are closely associated with cytokinin levels in the endosperms. Root-derived zeatin and zeatin riboside play a pivotal role in regulating cell division activity in the endosperm.

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