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Effect of panicle removal on the cytokinin level in xylem and nitrogen uptake activity of rice

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To evaluate the role of cytokinin in the source-sink relationship, panicles of rice were cut from the stem at the panicle emergence stage. Xylem sap exudates was collected by stem cut method and the cytokinin concentration in collected sap was determined by a bioassay and further analysis by ELISA. The rate of cytokinin translocation from roots to shoots decreased continuously after panicle initiation, whereas, when the panicle was removed, the rate increased by up to 1.5 times, at which time no cytokinin was found in the plants with panicles. As the retardation of leaf senescence was not observed and nitrogen concentration in the leaves continued to decrease after panicle removal irrespective of cytokinin (mainly dihydrozeatin riboside and trans-zeatin riboside) level. Leaf autonomy is thus regulated by an endogenous program of nitrogen translocation from the leaf regardless of cytokinin level in the xylem.

Key Words: cytokinin, nitrogen, panicle removal, rice, source-sink relationship

Abbreviations: BAeq, benzyl adenine equivalents; DHZ, dihydrozeatin; DHZR, dihydrozeatin riboside; ΔN, nitrogen uptake rate; iP, iso-pentenyl adenine; iPA, iso-pentenyl adenosine; iPG, iso-pentenyladenine-9-glu**coside; RGR, relative growth rate; SAR_N, specific absorption rate of nitrogen; tZ, trans zeatin; tZR, trans zeatin riboside

Cytokinins have been considered as signal compounds that provide signals from the roots to the shoots and function by directing information on the absorption of nitrogen by the roots (Sakakibara et al. 1998), the level of cytokinin decrease when plant are starved of nitrogen and *in versa* (Samuelson and Larsson 1993; Takei et al. 2001, 2002). Cytokinins are known to be synthesized mainly in the root tips, and are translocated to the shoot meristematic cells to enhance growth (Bernier et al. 1977) and retard leaf senescence (Gan and Amasino, 1995; Buchanan-Wollaston et al. 2003). While many plant parts are also known to have the ability to produce cytokinin (eg. Letham 1994), the major place of production is considered to be root. As nitrogen is absorbed through the roots, the focus has been on the role of cytokinins. The nitrogen uptake rate per unit root dry weight has been used as an indicator of root activity (Shinano et al. 1994; Osaki et al. 1996). In rice, it has been shown that keeping the specific absorption rate of nitrogen (SAR_N) high after flowering leads to higher productivity (Osaki et al. 1996). Comparisons of high-yielding and standard-yielding varieties have shown a higher retention of nitrogen and chlorophyll content in leaves in the former (Soejima et al. 1992; Osaki et al. 1993; He et al. 2002),

especially in the flag leaf (Soejima et al. 1995). By comparing two cultivars of different yield potential, Soejima et al. (1992) demonstrated a higher level of cytokinins in root exudates of the high-yielding cultivar. These researchers concluded that the retardation of leaf senescence observed in high-yielding cultivars was causally related to the higher level of cytokinins in the xylem sap exudate. That is the level of cytokinin is considered as a determinant factor for the regulation of leaf senescence. The relationship between the level of cytokinin in the leaves and leaf senescence has been studied in leaves of soybean at different ages (Singh et al. 1992) and by directly painting cytokinin onto the leaves of *Nicotiana rustica* (Mothes and Engelbrecht 1961) and of soybean (Garrison et al. 1984). Smart et al. (1991) constructed a transgenic tobacco that over-expressed iso-pentenyl transferase, which catalyzes the synthesis of the cytokinin precursor iso-pentenyl adenine riboside. The level of endogenous cytokinin in the transformant was 10 times higher than that in the untransformed plant, and leaf senescence was retarded in the transgenic plant. He et al. (2005) compared hormonal concentration of difference senescence types of maize, and found that the concentration of cytokinins (trans-zeatin riboside (tZR), dihydrozeatin riboside (DHZR), and iso-pentenyl adenosine (iPA)) were higher in stay green type.

When panicles were removed from rice plants at the flowering stage, the levels of rubisco, chlorophyll and cytochrome f remained higher than normal, and sucrose phosphate synthase and ADP-glucose pyrophosphorylase activities were also higher than normal (Nakano et al. 1995). Thus, the potential carbon-synthesizing activity of these plants could also be high. Osaki et al. (1995) reported that, in cereals, the removal of a sink (panicles) caused dry matter to accumulate in stems and thus plant productivity did not decrease as a result of sink removal. Thus at least in the case of rice plant, sink removal does not affect on the leaf photosynthetic traits and carbon allocation. We evaluated the effect of a reduced sink size (panicle removal) on the cytokinin levels in root exudates and investigated the relationship between the cytokinin level and nitrogen uptake ability and leaf senescence retardation in rice.

MATERIALS AND METHODS

Experiment 1. Effect of panicle removal on sap exudation, cytokinin content and nutrient uptake

Cultivation methods. The rice (*Oryza sativa* L.) cultivar Michikogane was cultivated in a paddy field at Hokkaido University, Japan. Seeds were sown on 19 April 2002 and transplanted on 30 May. Planting density was 30 cm x 15 cm and two seedlings were

planted per hill. One hundred kilograms N, 100 kg P_2O_5 as superphosphate and 100 kg K_2O were applied, with half of the nitrogen being applied as a slow-release fertilizer (Long 70, Chisso Asahi, Japan) and the other half applied as ammonium sulfate.

Panicle removal treatment was performed after 4 August at the panicle emergence stage, and treated plants were then checked at two-day intervals to remove panicles that appeared later.

Four to six plants were sampled at two-week intervals, and separated into live leaves, dead leaves, stems (including sheaths) and panicles (if present). Each sample was dried in an air-forced oven for at least 48 h at 80˚C, then weighed, ground and passed through a 1-mm mesh for subsequent analysis. To obtain roots, soil to a depth of 20 cm was sampled from two hills, and soaked in tap water overnight to make washing easier. The roots were washed with tap water and collected on a 2-mm-mesh net. Roots were also dried and ground. Three replications were conducted for each sampling.

Relative growth rate (RGR) was calculated as 1/W x dW/dt, where W is the dry weight of the plant (g) and t is the time (d). The specific absorption rate of nitrogen (SAR_N) was calculated as $\Delta N \times RW^{-1}$, where ΔN is the nitrogen absorption rate per day of the whole plant (g N m^{-2} d^{-1}) and RW is root dry weight (g m^{-2}). To calculate RGR, ΔN, SAR_N, as the experiment was done by using one field, all the data obtained at each sampling was averaged and used for the calculation to follow the subsequent changes in dry weight and accumulated amount of nitrogen.

Chemical analysis. Nitrogen concentration was determined by the semi-micro Kjeldahl method with three replications for each sample (Hind 1993).

Collection of xylem sap. Xylem sap exudate was collected overnight from 2000 to 0800 hours with five replications. The collection was performed only when the weather was clear. Xylem sap was collected by placing de-fatted cotton on the cut surface area of the rice base (about 10 cm in height from the top of the water level), which was then wrapped with a polyethylene bag and covered with a paper bag to avoid desiccation of the cotton. The amount of root exudate was determined by the change in weight of the cotton. Diurnal change of the cytokinin is known in barley (Kurapov et al. 2000), carrot (Paasch et al. 1997), and tobacco (Nováková et al. 2005). As the cytokinin level was rather stable

than during the day time, and also after the panicle was removed the effect of light condition on the cytokinin level in this experiment seems negligible.

Separation of cytokinins. Collected sap was recovered from the cotton by using 100% ethanol, and dried with a rotary evaporator at 40˚C, then resuspended in 1 mL of distilled water. After centrifugation at 16 300 g for 7 min at 4˚C, the supernatant was applied to a Sep-Pak C18 cartridge column (Waters Co. Ltd, Japan), which was pretreated with 99% methanol, followed by distilled water. The column was washed with 1 mL of distilled water and 20 mL of 55% methanol was added to elute the cytokinins. The eluted solution was evaporated, then dissolved in a phosphate buffer (pH 6.3, 1/60 M). This solution was used for the subsequent bioassays of total cytokinin activity.

Bioassay of cytokinin activity. Combined extracts were purified as described in Soejima et al. (1992). Cytokinin activity was determined by the Amaranths beta-cyanine bioassay as described by Biddington and Thomas (1973). *Amaranthus tricolor* cv. bicolor (commercial name Flying Colours) was used and cytokinin activity in the sample was compared with amaranths treated with benzyl adenine. Seeds of amaranth were germinated in a glass Petri dish (21 cm diameter) with three sheets of filter paper that were wetted with water (No. 2, Advantec Toyo, Tokyo, Japan). The glass Petri dish was placed in the dark at 27˚C for 48 h, then for 27 h at 32˚C. The seed coat and roots were removed from germinated plants to give a plantlet of about 1 cm length, consisting of the cotyledon and stem. A sample extract (0.4 mL) obtained as above and 0.1 mL of tyrosine solution (2.5 g L^{-1}) were mixed and then placed in a plastic Petri dish (3.5 cm diameter). One sheet of filter paper (No. 2, Advantec Toyo) was placed in the plastic Petri dish and then 18 plantlets were added. After 18 h at 27˚C under dark conditions, the plantlets were transferred to a polypropylene tube with 3 mL of distilled water. To extract beta-cyanine from the plantlets, the polypropylene tube was frozen and thawed seven times, and then the difference in the absorbance at 620 nm and 542 nm was determined. To obtain a standard curve, 5×10^{-8}, 1×10^{-8}, 5×10^{-7}, 1×10^{-7}, 5×10^{-6} and 1×10^{-6} M solutions of benzyl adenine were made up with phosphorus buffer (pH 6.3, 0.017 M). Although it is difficult to determine the exact cytokinin concentration by bioassay, it can be useful in comparing the same species when using the same collection method. Data were expressed as benzyl adenine equivalents (BAeq).

Fractionation of cytokinins. Purified cytokinins were separated by high performance liquid chromatography (HPLC) using the methods described by Soejima et al. (1995). Samples were collected from the control treatment (those plants whose sinks were not removed) and the sink removal treatment on 29 August (at mid-maturation stage). After being concentrated as described above, each cytokinin fraction was separated by HPLC (D-ODS-10-A, 250 mm x 20 mm; YMC, Kyoto, Japan) with 50% methanol at 6.0 mL min^{-1}. trans-Zeatin riboside (tZR), zeatin (Z), iso-pentenyl adenosine (iPA) and iso-pentenyl adenine (iP) were used as standard compounds to determine the retention times of cytokinins. These were 16.4, 18.11, 50.79 and 57.18 min, respectively. Thus, cytokinins were separated into six fractions, as Rt: 0–14.0, 14.0–17.4, 17.4–22.0, 22.0–47.0, 47.0–54.5 and 54.5–65.0 min. Each fraction was considered to contain conjugated cytokinins, tZR (and/or dihydrozeatin riboside (DHZR)), tZ (and/or dihydrozeatin (DHZ)), isop-entenyladenine-9-glucoside (iPG), iPA, and iP, respectively.

Experiment 2 Determination of plant hormones by ELISA

Cultivation method. In 2003, to confirm the effect of sink removal on the composition of plant hormones in the bleeding sap, another experiment was performed. The rice cultivar Michikogane was cultivated in a 12 liter pot with the soil obtained from the paddy soil. Nitrogen as ammonium sulfate, phosphorus as superphosphate and potassium as potassium sulfate were fertilized as 1.5g N, 1.5g P_2O_5, and 1.5g K_2O per pot. Seeds were sown on 28 April 2003 and transplanted on 3 June. Panicle removal treatment was performed on 7 August at the panicle emergence stage, and treated plants were then checked every day to remove panicles that appeared later. Sap was collected as described above on 5th to 6th September with 5 replications.

Extraction of hormone and quantification. Cytokinins are further analyzed for those compounds known to have high activity; tZR, DHZR and iPA were tested. The methods for extraction and purification were modified from those described by He et al. (2005). After sap was collected and evaporated as described in Experiment 1, dissolved in 3 ml of 80% MeOH. The extracts were purified by passing them through Sep-Pak Plus C18 cartridge (Waters, Milford, USA). The cartridge was previously eluted with 10 ml of 99% MeOH, then passed 5 ml of 80% MeOH. After sample was put into cartridge the cartridge with syringe, 2ml of 80% MeOH was used to wash the cartridge. Combined elutes were evaporated and dissolved in 1.0 ml of TBS buffer (tris-buffered saline; 50 mM

Tris, pH 7.8, 1 mM $MgCl_2$, 10 mM NaCl, 0.1% tween, 0.1% gelatin). DHZR, tZR and iPA contents were determined by ELISA using monoclonal antibodies (Phytodetek, Agdia, Elkhart, IN, USA) following the protocol provided by the manufacturer. As the antibodies of cytokinins also recognize free bases, nucleotides and 9N-glucosides, what was measured was the sum of free basis, ribosides, nucleotides and 9N-glucosides of corresponding cytokinins. The amount of DHZR also includes DHZ and tZR includes tZ.

RESULTS

Plant growth

Panicle emergence occurred on 4 August (66 d after transplanting) and harvest was on 4 October (122 d after transplanting). In the panicle removal treatment, the number of tillers increased from 500 tillers m^{-2} to 1100 tillers m^{-2}, whereas, in the controls, the number of tillers decreased gradually to 450 tillers m^{-2} (data not shown). However, the new tillers in the panicle removal treatment contributed little to the dry matter or nitrogen nutrient status of the plant because of their small size.

The dry weight of the whole plant increased exponentially until the panicle emergence stage, then increased gradually until two weeks before harvest (Fig. 1). When the sink was removed, the increase in dry weight stopped temporarily and then increased again. In the panicle removal treatment, the dry weight of the stem increased and the leaf dry weight remained constant until harvest (Fig. 1).

The root dry weight increased until 108 d after transplanting and was not affected by removal of the sink (Fig. 1).

Accumulation of nitrogen

Nitrogen accumulated in the leaves until the panicle emergence stage, then decreased regardless of treatment (Fig. 1). More nitrogen accumulated in the stem in the panicle removal treatment than in the control after the panicle emergence stage, indicating that the stem has a role as an alternative nitrogen sink.

After panicle emergence, nitrogen increased in the control, whereas the accumulated nitrogen gradually decreased when the panicles were removed (Fig. 2).

Nitrogen and chlorophyll concentration

Nitrogen concentration in the leaves decreased rapidly after the panicle emergence stage (Fig. 2). The chlorophyll concentration increased until 88 d after transplanting, then

decreased rapidly (Fig. 2). There was no difference between the control and the panicle removal treatment in the concentrations of either nitrogen or chlorophyll. The nitrogen concentration in the roots decreased gradually until the mid-maturation stage, when the concentration increased. Nitrogen concentration in the roots increased at harvest if the panicle was removed (Fig. 2).

Nitrogen uptake and specific absorption rate of nitrogen

The nitrogen uptake rate (ΔN) increased until 48 d after transplanting, and then decreased. The uptake rate was suppressed by panicle removal. The specific absorption rate of nitrogen (SAR_N) showed a tendency similar to ΔN (Fig. 3). SAR_N also decreased in the panicle removal treatment.

Cytokinin translocation rate and xylem sap exudation rate

The cytokinin translocation rate increased until the booting stage, and then decreased quickly. When the panicle was removed at the panicle emergence stage, the cytokinin translocation rate increased rapidly for about five weeks and then decreased rapidly after the mid-ripening stage (Fig. 4).

The exudation rate of xylem sap was high before flowering, and then decreased sharply during the reproductive stage (Fig. 4). When the panicle was removed, the xylem sap exudation rate became higher than that in the control treatment.

Composition of cytokinins in the xylem sap

The effect of sink removal on the composition of cytokinins is shown in Fig. 5. The cytokinin activity was mainly derived from a large amount of activity occurring in chromatographic fractions where ZR, tZ and iPG standards occurred. This activity may correspond to ZR, tZ and iPG, but definitive identification will require MS analysis with heavy isotope labeled standards or other critical identification techniques.

Determination of cytokinins by ELISA

In iPA, DHZR and tZR, a significant increase was observed by sink removal in the level of tZR (Table 1). Though the level of iPA decreased by the sink removal, the change was not significant.

DISCUSSION

Leaf nitrogen status and cytokinin

Sink activity is sometimes thought to be detrimental to the regulation of the balance of the source-sink. Dry matter production is assumed to be regulated by the activity of the sink on the basis of the following results: 1) when the sink was removed, the photosynthetic rate decreased markedly in wheat (King et al. 1967), and 2) when varieties with different source-sink relations were grafted, the photosynthetic rate varied with the strength of the sink in sweet potato (Hahn 1977) and in beet (Thorne and Evans 1964). However, in recent studies, photosynthetic rate or dry matter production of whole plant did not decrease, even after the sink organs (panicles in cereals and pods in legumes) were completely removed (Koide and Ishihara 1992a, b; Osaki et al. 1995). Osaki et al. (1995) observed that nitrogen translocation from the leaves of various cereal crops was not affected by panicle removal. This observation indicates that the destruction and translocation of nitrogen compounds in leaves was not regulated by sink demand, but that there was a physiological autonomy in the leaves. In the current paper, leaf autonomy in terms of nitrogen translocation during the ripening stage of the rice plant was confirmed, because 1) the nitrogen and chlorophyll concentrations in the leaves were not affected by panicle manipulation (Fig. 2), and 2) even in the panicle removal treatment, nitrogen was seen to decrease in the leaves, then either accumulate in the stems or be lost from the plants entirely (Fig. 1). This indigenous regulation of nitrogen translocation from the leaves is observed in cereals, but, in legume crops, leaf senescence and nitrogen translocation from the leaves are depressed by sink manipulation (Noodén and Letham, 1993; Osaki et al. 1995).

Exogenous application of cytokinin is known to delay leaf senescence by retarding the degradation of chlorophyll and photosynthetic proteins (Badenoch-Jones et al. 1996). Genetic manipulation of tobacco to produce cytokinin under the control of a senescence-driven promoter revealed that the expression of cytokinin in the tobacco leaves could delay leaf senescence (Gan and Amasino 1995). The production of cytokinin also prolonged the photosynthetic activity of tobacco (Wingler et al. 1998; Jordi et al. 2000). Cytokinin is known to up-regulate several genes such as the rubisco small subunit (Plumley and Schmidt 1991), and phosphoenolpyruvate carboxylase (Sugiharto et al. 1990; Sugiharto and Sugiyama 1992). It has been reported that, under nitrogen-limiting conditions, exogenously applied nitrogen could induce several genes that regulate photosynthesis in maize, and this induction can occur with the addition of cytokinin (Suzuki et al. 1994).

The pattern of decreasing chlorophyll concentration in the leaves did not change, and thus leaf senescence was unaffected, regardless of the increase of the cytokinin level in the xylem sap (Fig. 4). In general, it is assumed that leaf senescence is regulated by sink demand (panicle formation) and cytokinin concentration; however, at least in rice plants, the data obtained suggest that leaf senescence is not regulated by its endogenous program, and not by sink (panicle) demand for nitrogen nor by cytokinin level directly (Fig. 2). From the comparison between different senescent type maize, He et al. (2005) demonstrated that the stay-green hybrid showed more vigorous root growth and cytokinin production than early senescent hybrid. Furthermore, though t-ZR and DHZR contents are larger in the root of stay-green hybrid than early senescent hybrid, that of iPA was in versa. As the similar situation of stay-green hybrid was observed in panicle removal treatment, it is suggested that panicle removal supports a large distribution of carbohydrates from leaves to roots makes the root activity for the synthesis of cytokinins kept high. While the disturbance of source-sink balance by panicle removal changed the composition of cytokinins.

Nitrogen demand and absorption

Nitrogen uptake was stopped by the panicle removal treatment (Fig. 3), thus the uptake of nitrogen by the roots seemed to be regulated by the sink requirements regardless of carbohydrate status. Accordingly, nitrogen absorption is regulated by a strong nitrogen requirement. High root activity is important for achieving high productivity; however, the nitrogen absorption activity of the roots must relate to a high nitrogen demand in the shoot. On the other hand, as the root nitrogen concentration increased and significantly higher in panicle removal treatment than that of control (Fig. 2), which indicates retardation of nitrogen transport occurred by panicle removal.

Xylem sap exudation

It is widely accepted that cytokinins are root-produced phytohormones and that they are transported to the shoot through the xylem. While the expression of genes encoding the synthesis of cytokinin (ATP/ADP iso-pentenyl transferases) clearly demonstrated that the expression occurred in a wide range of organs and cell types (Miyawaki et al. 2004). Furthermore, it should be noted that the obtained cytokinin in this experiment is not solely derived from xylem, as the occurrence of phloem to xylem transport of cytokinin is reported (Emery et al. 2000), furthermore the contribution of

rhizobacteria which produce cytokinin and transported into plant is also known (Garcia de Salamone et al. 2001). We do not know the contribution of phloem and/or rhizobacteria derived cytokinin in this experiment, while in the latter case it is suggested that if carbohydrate supply from shoot to roots increased after sink removal and which promotes the production of cytokinin by rhizobacteria it can be incorporated in the role of cytokinin from the root. If more cytokinin was transported from the roots to the shoot, the vigor of the shoot can be assumed to remain high. After panicle removal treatment, a large increase in the amount of xylem sap exuded indicating the higher activity of root and with the increment of several cytokinin in the xylem sap can be contributed (Fig. 4, Table 1). Though we have considered two opposite situations on root activity by panicle removal treatment, as the amount of xylem sap and cytokinin translocated in the stem increased by panicle removal, it is suggested that the root activity seems to be supported or impressed. However, as dry matter increase and nitrogen uptake decreased by panicle removal treatment, nitrogen uptake by roots was differently regulated. A large increase of cytokinin by panicle removal seems to make new destination of photoassimilates, and this may increase the number of tillers of rice by panicle removal (data not shown), but it was in vain in the meaning of panicle production. The cytokinin concentration in xylem exudate increased after decapitation of the bean crop (Bangerth 1994). Although cytokinin has recently been considered to be a signal compound that responds to nitrogen in maize roots (Sakakibara et al., 1998), pod removal of soybean (Noodén et al., 1990) and rice (this report) or decapitation in the bean (Bangerth 1994) also induce a large increase of cytokinin in the stem exudate. Cytokinin biosynthesis seems to be regulated by more than just nitrogen and the observed leaf autonomy for nitrogen was not affected by the increased level of cytokinin. This result is also supported by the study of cytokinin deficient Arabidopsis (Werner et al. 2003), where the mutant did not show any promotion of senescence.

Changes of cytokinin composition by panicle removal

In this study, cytokinin activity in the root exudates was separated into different types of cytokinin fraction by using HPLC. Murofushi et al. (1983) reported the existence of cis-zeatin, cis-ribosyl zeatin, tZR and zeatin glucoside (conjugated fraction) in rice root exudates. Soejima et al. (1992) reported the existence of N^6-isopentenyladenosine,tZR, tZ and conjugated zeatin, with conjugated zeatin appearing in the highest amount in rice. Thus, these researchers judged conjugated zeatin to be important in cytokinin transport

from roots to shoots in rice. In the control plants, results from our chromatographic fraction suggest a similar tendency. Though the detailed cytokinin related compounds might be evaluated by using other techniques (eg. LC-MS), it is still important to consider the major cytokinin and bioassay for evaluating the role of cytokinin in the plant.

In this report, the presence of tZR, tZ and iPG in the exudates of control plants was suggested by chromatographic behavior. After removing the panicles, we observed an increased activity not only in the tZR and tZ fractions, but also in the iPG fraction. Though the increase in the concentration in the xylem sap of active form of cytokinin is only observed in be tZR from ELISA (Table 1), as the amount of xylem sap exudation increased significantly by the panicle removal all of the amount of active cytokinin forms (iPA, tZR, DHZR) increased significantly (Table 1). It is suggested that panicle removal affect over all expression of cytokinins synthesis. Although glucosylated cytokinins are categorized as inactive, some have shown high activity, as was observed in oat, radish, Chinese cabbage (Letham and Palni 1983), sweet potato (Sugiyama and Hashizume 1989; Sugiyama et al. 1983) and petunia (Auer and Cohen 1993). The change of relative strength of RZ, tZ and iPG fraction to iPA and iP fraction from the data of HPLC, or the relative strength of DHZR and tZR to iPA from the data of ELISA, it is suggested that these ratio could be a result of source-sink balance change in rice plant. As recent analytical development to determine the cytokinin metabolite more precisely (eg. Nováková et al. 2005) and not only the synthesis of the cytokinin but degradation of cytokinin by cytokinin oxydase/dehydrogenase also play an important role for the regulation of rice productivity (Ashikari et al. 2005), further study is required to determine why the cytokinin composition is changed by the panicle removal treatment.

Though recent findings clarified cytokinin signaling within a cell (eg. Howell et al. 2003), and the importance of cytokinin on the regulation of shoot development is widely accepted (eg. Scmülling 2001). However, though the cytokinin level in the xylem sap was changed by the panicle removal, any retardation of leaf senescence was not observed in the present experiment. Which indicates that the actual role of cytokinin seems different at cellular level and individual level. If we consider to use cytokinin for the regulation of rice plant growth, it is required to understand long distance cytokinin signaling more precisely.

CONCLUSION

Panicle removal of rice plant resulted a large increase of cytokinin level (both in the amount and activity) in the xylem sap. While the leaf senescence was not delayed from the

viewpoint of nitrogen and chlorophyll concentration change. Thus, it should be noted that leaf senescence is not regulated by a single attempt of cytokinin level, but the role of cytokinin on the leaf senescence seems to be changed by the plant (or leaf) developmental stage also. These findings are especially important to consider the role of cytokinin on the rice plant growth regulation.

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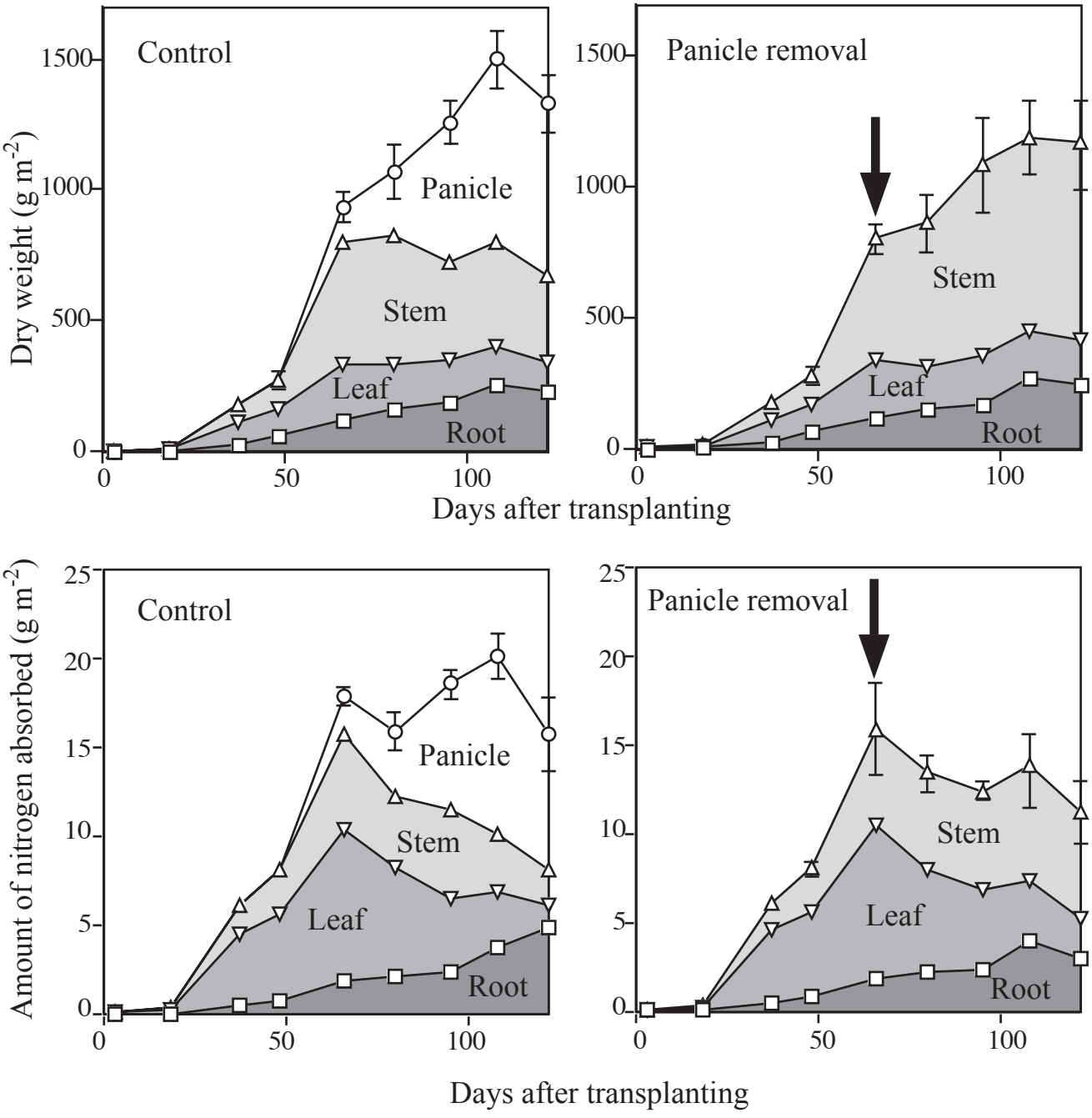

Fig. 1 Successive changes in dry weight and in the amount of nitrogen absorbed in various parts of the plant. Data are cumulatively accumulated and the top valueis equal to the whole plant dry weight. Bars in the figure indicate SE (n = 3) of the whole plant dry weight, those symbols without bars indicate the SE was small enough to be covered under the symbol. Arrows in the figure indicate the initiation date of panicle removal.

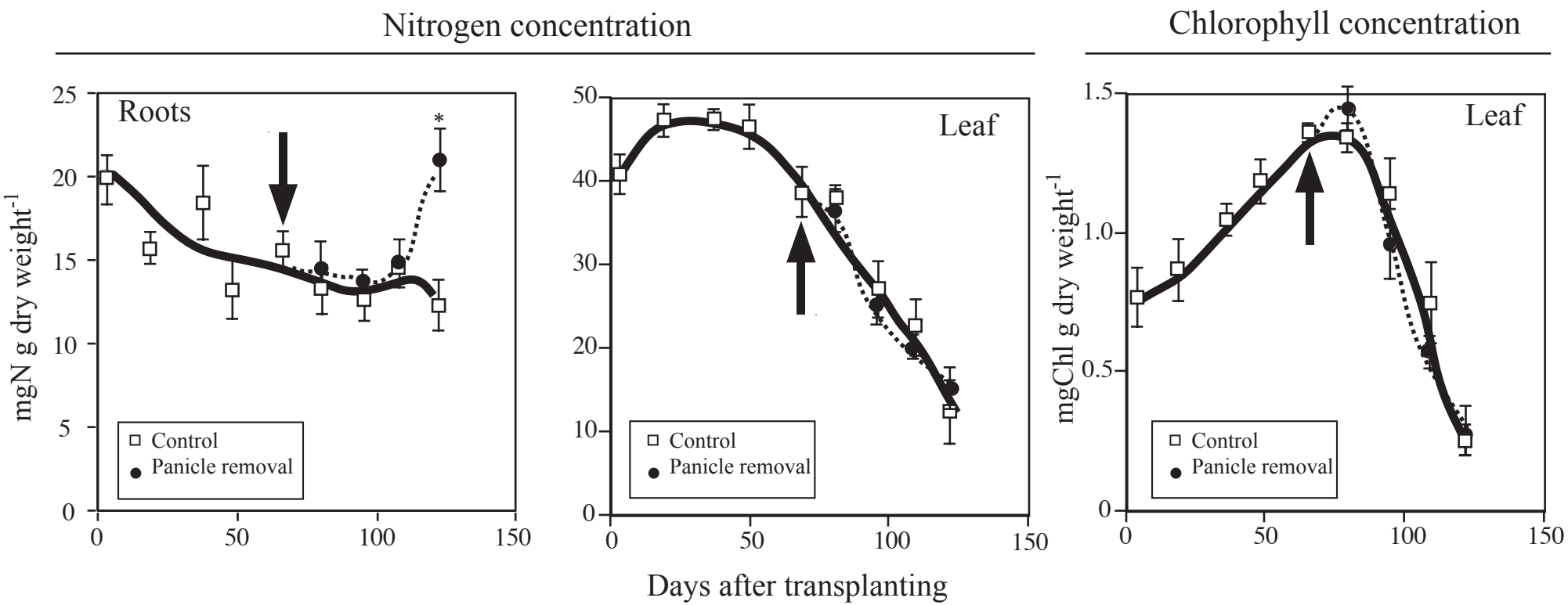

Fig. 2 Successive changes in root and leaf nitrogen concentration and in the chlorophyll concentration in green leaves of plants treated to remove the panicle compared with an untreated control. Arrows in the figure indicate the initiation date of panicle removal. Bars in the figure indicate SE. Asterisk on the bar indicates the difference between control and panicle removal is significantly different ($p < 0.05$) by t-test.

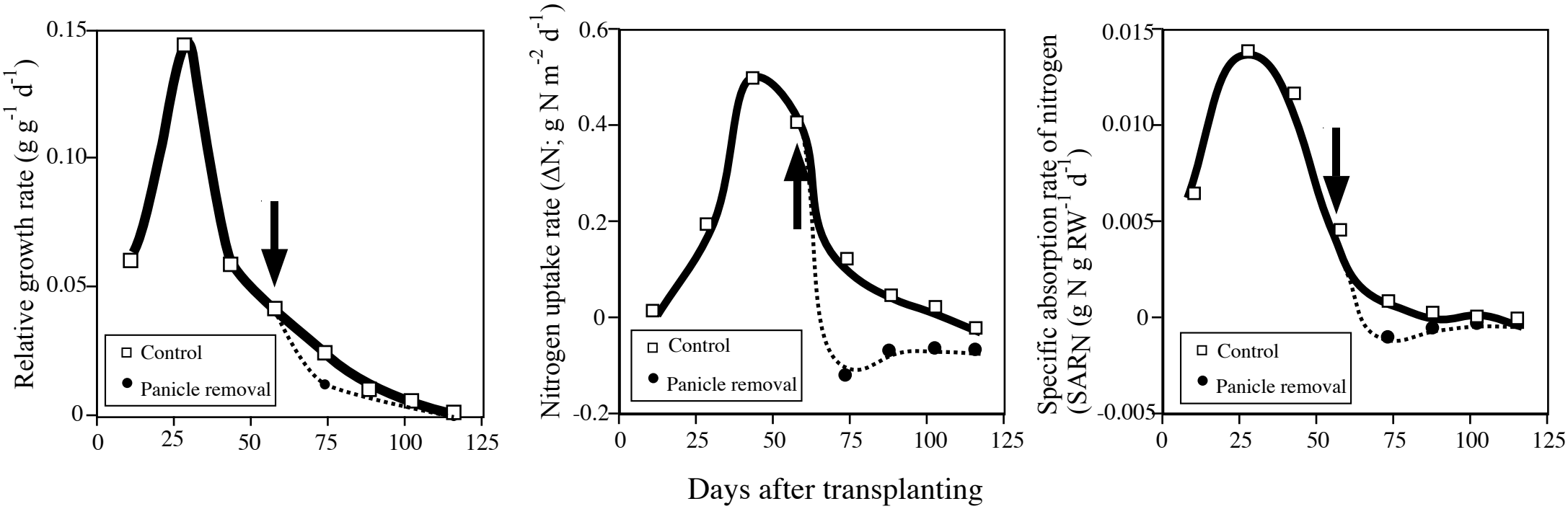

Fig. 3 Successive changes in the relative growth rate (RGR; g g^{-1} d^{-1}), nitrogen uptake rate (ΔN; g N m^{-2} d^{-1}) and specific absorption rate of nitrogen (SAR_N; g N g RW^{-1} d^{-1}). To calculate RGR, ΔN, SARN, as the experiment was done by using one field, all the data obtained at each sampling was averaged and used for the calculation to follow the subsequent changes in dry weight and accumulated amount of nitrogen, so the data are without replicate. Arrows in the figure indicate the initiation date of panicle removal.

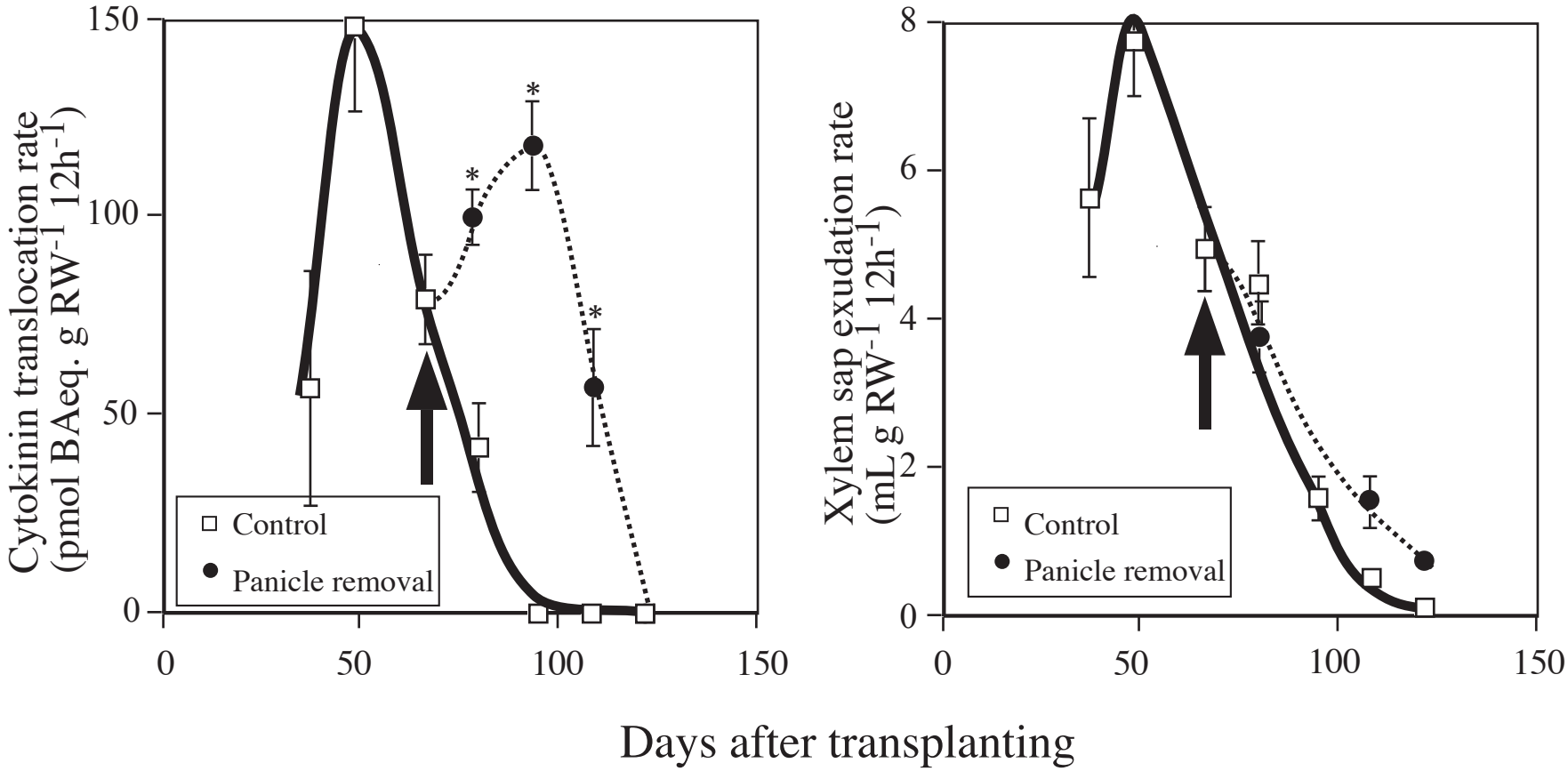

Fig. 4 Successive changes in the xylem sap exudation rate (mL g RW^{-1} $12h^{-1}$) and the cytokinin translocation rate (pmol BAeq. g RW^{-1} $12h^{-1}$). The cytokinin level is indicated as benzyl adenine (BA) equivalents. Arrows in the figure indicate the initiation date of panicle removal. Bars in the figure indicate SE, those symbols without bars indicate the SE was small enough to be covered under the symbol. Asterisk on the bar indicates the difference between control and panicle removal is significantly different ($p < 0.05$) by t-test.

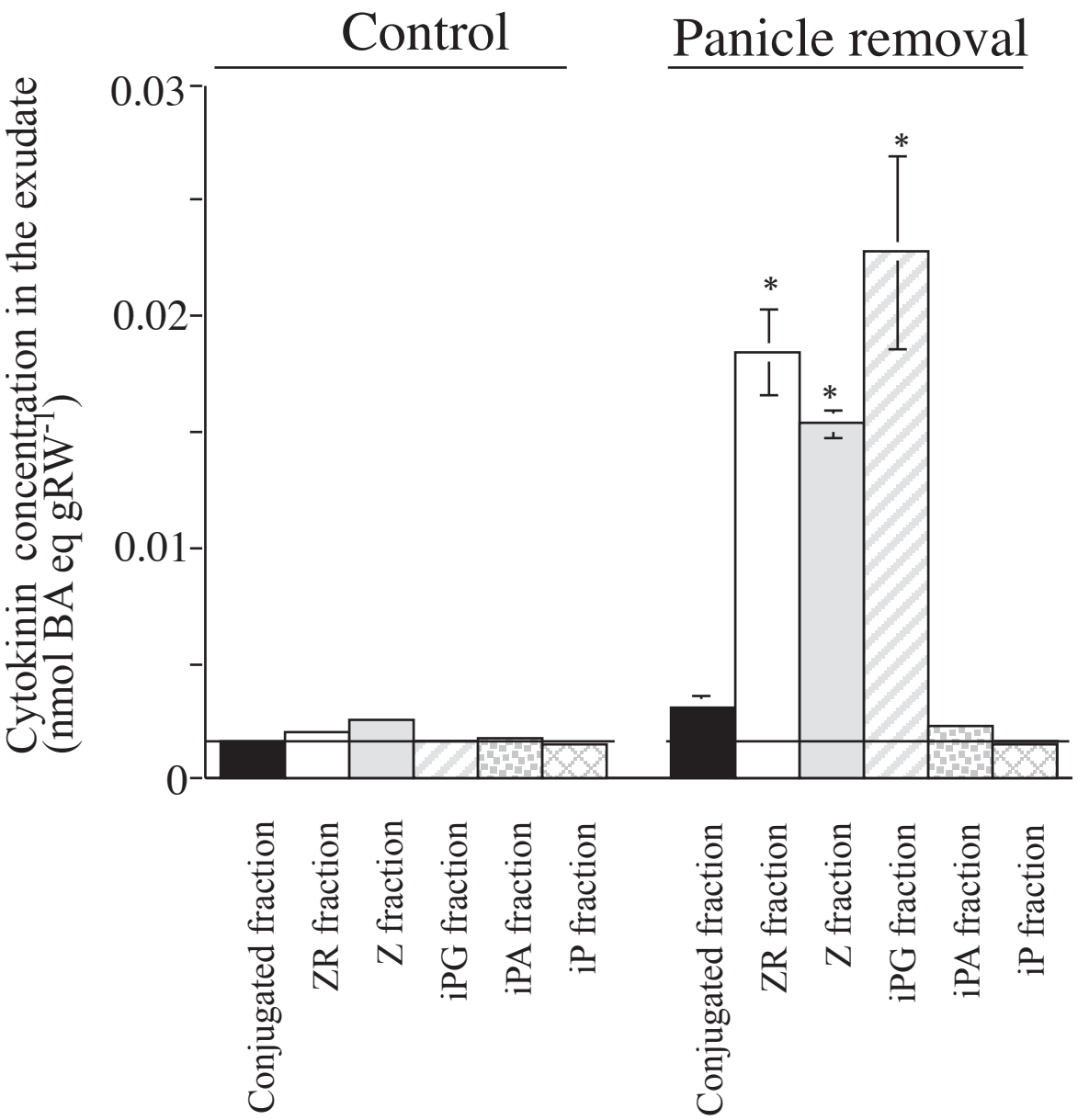

Fig. 5 Cytokinin activity by the Amaranthus beta cyanine bioassay in fractions separated by HPLC and identified by standards that separated into each fraction. Bars in the figure indicate SE, those symbols without bars indicate the SE was small enough to be covered under the symbol. Asterisk on the bar indicates the difference between control and panicle removal is significantly different ($p < 0.05$) by t-test.

Table 1 Concentration of cytokinins determined by ELISA method.

		Cytokinins content (pmol/sap(g))		
	Sap amount (g)	iPA	DHZR	tZR
Control	5.76±0.86	124.8±30.0	3.83±0.68	1.87±0.22
Panicle removal	16.05±1.37	81.5±13.0	3.47±0.85	4.54±0.93
Difference	1%	NS	NS	5%

	Cytokinins amount in sap (pmol/gRW/12hr)		
	iPA	DHZR	tZR
Control	4.25±0.83	0.13±0.02	0.07±0.01
Panicle removal	9.19±1.39	0.38±0.09	0.51±0.09
Difference	5%	5%	1%