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Ethylene inhibitors enhanced sucrose synthase activity and promoted grain filling of basal rice kernels

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Abstract. At the booting stage of development, rice (*Oryza sativa* L.) plants were treated with chemicals that either inhibited the action or synthesis of ethylene, or produced ethylene. Inhibitors of ethylene action ($AgNO_3$) and synthesis [uniconazole, paclobutrazol, $Co(NO_3)_2$] promoted grain filling and quality of the kernels of the basal spikelets of the panicle, while the ethylene-releasing substance CEPA (2-chloroethylphosphonic acid) depressed these characteristics further. The inhibitors depressed the concentration of ethylene of the basal primary branches, but CEPA increased it above the control during the period of grain filling. The treatments were not effective on the superior apical spikelets of the panicle. The ethylene inhibitors improved starch synthesis in the kernels of the basal spikelets, but CEPA reduced it significantly, resulting in accumulation of soluble carbohydrates in the kernels. During the period of grain filling, sucrose synthase activity was higher than that of invertase in the kernels. Activities of sucrose synthase and invertase were higher in the apical than in the basal kernel. The ethylene inhibitors increased activities of both enzymes only in the basal kernel, whereas CEPA reduced activities significantly. Together, the results indicate that starch filling and grain quality of the basally positioned under-developed rice kernels can be affected by ethylene, and that key enzymes of sucrose metabolism are also affected in the process.

Keywords: CEPA, ethylene inhibitors, grain filling, rice, spikelet, sucrose synthase.

Introduction

A rice (*Oryza sativa* L.) panicle is composed of a large number of spikelets, and each spikelet is genetically competent to produce high-quality grain at maturity. However, most of the spikelets found on the proximal primary branches of the panicle are poor in growth and development, and do not bear high-quality grains suitable for human consumption (Mohapatra *et al.* 1993). In contrast, the high-quality spikelets of the apical primary branches grow faster to achieve higher final dry weight and produce high-density grains at maturity in comparison to the inferior grains of the proximal branches. The capacity for starch synthesis in the endosperm cells of the inferior spikelets is poor (Umemoto *et al.* 1994), and assimilates partitioned to them remain unused (Patel and Mohapatra 1992; Mohapatra *et al.* 1993). Inside the developing caryopsis, sucrose is the major phloem solute. Sucrose is unloaded from the maternal seed coat into the embryonic apoplast surrounding the endosperm and embryo (Thorne 1985), from where it is actively transported into the endosperm (Oparka and Gates 1981*a*, *b*). In the endosperm, sucrose is converted into starch via two pathways initiated by either sucrose synthase or invertase (Perez *et al.* 1975; Nakamura and Yuki 1992; Morrell *et al.* 1995). Activities of these enzymes are low in the endosperm of under-developed basal spikelets, which ultimately reduces starch synthesis and grain filling (Patel and Mohapatra 1996).

Although most of the sucrose-importing plant cells use both enzymes for cleavage of sucrose for starch synthesis (Morris 1996), sucrose hydrolysis mostly occurs by sucrose synthase, and invertase plays a minor role in the process (Wang *et al.* 1993; Kato 1995; Riffkin *et al.* 1995; Patel and Mohapatra 1996). Evidence from wheat (Dale and Housley 1986) and rice (Patel and Mohapatra 1996) indicates that grain growth at unfavourable positions on the inflorescence (inferior grains) is not limited by shortage of assimilates, but by the activity of the enzyme sucrose synthase. A similar observation is made in heat-stressed barley, where the effect of stress on sucrose synthase activity reduced starch synthesis and grain growth leading to accumulation of assimilates (McLeod and Duffus 1988).

The mechanism by which endogenous growth hormones regulate dry matter partitioning into rice grains has received little attention. Lack of knowledge on this vital aspect of grain filling has precluded manipulation of dry matter partitioning in favour of the inferior basal spikelets by exogenous application of chemical growth regulators. Japanese research

Abbreviations used. ABA, abscisic acid; ACC, 1-aminocyclopropane 1-carboxylic acid; CEPA, 2-chloroethylphosphonic acid; GA, gibberellic acid; IAA, indole-3-acetic acid; PBZ, paclobutrazol; UCZ, uniconazole.

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(Kato and Takeda 1993; Kato *et al.* 1993) indicates that abscisic acid (ABA) promotes assimilate partitioning into the rice grains during grain filling. This conclusion was reached by comparing the ABA contents of superior grains located on the primary branches of the rice panicle with those of inferior grains on secondary branches in one variety, and grains of two rice varieties differing in grain size and weight during the period of grain filling. Such discoveries emphasise the need for application of growth inhibitors such as ABA for promotion of grain filling of the under-developed basal spikelets of the rice panicle.

On the other hand, application of some growth promoters to rice has shown results to the contrary. Applications of gibberellin and cytokinin have been reported to enhance development of these spikelets, while indole-3-acetic acid (IAA) depressed the development of the organs more than in the control (Patel and Mohapatra 1992). Similarly, inhibitors of ethylene synthesis and action improved the number of grain-bearing spikelets of rice panicles, and the ethylene-releasing substance 2-chloroethylphosphonic acid (CEPA) depressed the number significantly (Naik and Mohapatra 1999). The effects of ethylene inhibitors were mostly confined to the poorly developed basal spikelets. They improved development and dry matter partitioning of the spikelets, and ultimately increased grain yield of the panicle (Mohapatra *et al.* 2000). Such effects of ethylene inhibitors on basal spikelets suggest involvement of endogenous ethylene in inter-organ signalling between the dominant apical spikelets and the subordinated basal spikelets of rice panicle. The former may enjoy superiority in growth and development at the cost of the latter.

The role of ethylene in inter-organ signalling has been highlighted in the literature (Woltering *et al.* 1995; Bui and O'Neill 1998) and it will not be out of context to envisage its prominent role in the physiological dominance of the apical spikelets over their basal counterparts within the rice panicle. Since the apical spikelets develop faster and reach anthesis early compared to the basal spikelets, they may produce ethylene or its precursor to delay development of the latter. In the present experiment, an attempt has been made to regulate the action or synthesis of ethylene through the application of chemicals, and to assess their influence on the physiological processes responsible for grain filling.

Materials and methods

Plant material and culture

A high-yielding semi-dwarf rice cultivar (*Oryza sativa* L. cv. Lalat) was cultivated in cemented pots containing farmyard manure in open field conditions at the School of Life Science, Sambalpur University. Two experiments were performed, the first during the dry season (January–May) of 1998 and the second during the wet season (June–December) of 1999.

Seedlings grown in nursery beds were transplanted to 33 × 33 × 26-cm pots. In each pot, eight plants were grown in four hills (groups of two seedlings) with identical spacing. Commercial fertilisers, consisting of N (urea, Sindri Unit of the Fertiliser Corporation of India, New Delhi, India), P_2O_5 (single superphosphate, The Dharmsi Murarji Chemical Ltd, Mumbai, India) and K_2O (muriate of potash, Indian Potash Ltd, Chennai, India) were applied to the plants at the ratio of 80:40:40 in three split doses in the rooting medium. Half of each fertiliser was applied to the plants at the time of transplantation and one-quarter each at tillering and anthesis. The plants were free from any physico-chemical or biological stresses, and the pots were watered daily.

Chemical treatments

In both experiments, the pots were arranged in a randomised block design for six treatments with three replicates. $AgNO_3$ (10^{-5} M), $Co(NO_3)_2$ (10^{-5} M), 2-chloroethylphosphonic acid (10^{-5} M), paclobutrazol (100 μL L^{-1}) and uniconazole (0.5 mg L^{-1}) (a gift from Professor R. A. Fletcher, University of Guelph, Canada) were applied to the plants at the time of booting. The chemicals were dissolved in distilled water and 0.5 mL of the solution was injected carefully from the top into the boot of the flag leaf containing the emerging panicle with a 1-mL syringe. The sixth treatment consisted of control plants, which received distilled water only. The chemical treatments were given early in the morning after sunrise for four consecutive days with effect from the booting stage and discontinued thereafter.

Experiment I

Harvesting

In each treatment, plants were screened and marked for uniform growth and development, and samples were obtained from these plants. Five spikelets of the uppermost primary branch of the panicle, which reached the stage of anthesis on the first day, were collected from the panicle. These spikelets were called the 'apical' spikelets. Subsequent samplings of these spikelets were carried out at 5-d intervals up to the time of maturity. Similarly, five spikelets were collected from the two lowermost primary branches of the panicle when they reached the stage of anthesis; these were the 'basal' spikelets. Their sampling also continued up to maturity at 5-d intervals. The basal spikelets reached anthesis 5 d after the apical spikelets. On each occasion of sampling, three sets of samples were collected from the plants. In the first set, the kernels of the spikelets, bare of lemma and palea, were weighed fresh and kept in an oven at 90°C for 24 h for estimation of dry weight. The second set of spikelets (kernels only) was used for carbohydrate analyses, while the third was used for enzyme assays. At maturity, grain quality was measured in an extra set of plants, where all grains of the uppermost primary branch of the panicle and two lowermost primary branches were taken into consideration.

Extraction and estimation of carbohydrates

The kernels obtained from the apical and basal spikelets were weighed fresh, immersed immediately in 3 mL of boiling 80% aqueous methanol and boiled for 5 min. The extract was removed to a 10-mL volumetric flask. The residue was extracted a second time with 50% aqueous methanol, and both the extracts pooled. The volume of extract was made up to the mark with distilled water. Aliquots of the extract were used for estimation of total soluble sugars (Yemm and Willis 1954) and reducing sugars (Nelson 1944). The residue after methanolic extraction was digested with 3% HCl and used for the estimation of starch according to the method of Buysee and Merck (1993).

Sucrose synthase and acid invertase activities

Kernels (10 from both apical and basal spikelets) were homogenised separately in 5 mL of 50 mM Hepes buffer (pH 8.0) containing 8 mM $MgCl_2$, 2 mM EDTA, 50 mM 2-mercaptoethanol, 12.5% (v/v) glycerol and 5% (w/v) insoluble polyvinylpyrrolidone-40 in an ice-cooled mortar. The homogenate was centrifuged at 12 000 *g* for 15 min. The supernatant was collected in a 5-mL volumetric flask and the volume

was diluted to the mark with buffer. Aliquots of the stock solution were used for measuring sucrose synthase and invertase activities as described in Patel and Mohapatra (1996).

Measurement of grain quality

The mature grains of the panicle were immersed in a series of NaCl solutions of specific gravity varying between 1.0 and 1.2. The grains were classified according to their density: poor (floated at a specific gravity of 1.0–1.06), average (1.06–1.14), good (1.14–1.2) and high-density (submerged at a specific gravity of 1.2), according to the procedure of Venkateswarlu *et al.* (1986).

Experiment II

Harvesting

The uppermost and lowermost primary branches of the panicle were harvested from the main shoot of the plant by severing them from the panicle axis on the day the chemical treatments were administered first in each treatment. The fresh weights of the branches were recorded immediately after excision on an electronic balance. The cut ends of the branches were dipped into 1 mL of distilled water, separately in 15-mL glass test tubes. The test tubes containing the branches were sealed tightly with rubber caps and incubated in darkness for 2 h at room temperature.

Ethylene analysis

Headspace gas (1 mL) from the test tube containing primary branches was drawn into a gas-tight syringe and injected into a gas chromatograph (model 6890, Hewlett-Packard Company, Palo Alto, CA, USA) equipped with a flame ionisation detector. The micro-capillary column (length 30 m, internal diameter 0.53 mm) was packed with cross-linked methyl siloxane. Hydrogen and oxygen were used for the flame ionisation detector and nitrogen was the carrier gas. The detector and injector temperatures were 150 and 100°C, respectively. Subsequent harvestings and ethylene analyses were performed at 5-d intervals up to the time of maturity. The apical branch reached the stages of anthesis and maturity on d 5 and 32, respectively, after the chemical treatment. Anthesis and maturity of the basal branch were delayed by 5 and 3 d, respectively, in comparison to the apical branch.

Results

Experiment I

Grain quality

Most of the spikelets of the uppermost primary branch of the panicle produced high-density grains at maturity, and the chemical treatments did not influence grain density. Application of ethylene inhibitors improved grain density significantly in the spikelets of the proximal primary branches, and uniconazole was the most effective (Fig. 1). In contrast, CEPA application depressed grain density of these spikelets and increased the number of low-density grains. CEPA application advanced grain maturity on the proximal primary branches by 2 d, while ethylene inhibitors delayed maturity; a maximum delay of 3 d was observed in the uniconazole-treated plants.

Kernel growth

Dry mass of the kernels increased in a sigmoidal fashion with time in both apical and basal spikelets (Fig. 2). However, the rate of growth was much faster in the apical than the basal kernels, and the former accumulated nearly twice the biomass at maturity than did the latter. The chemical treatments did not change the dry mass of the apical kernel, but they were effective on the basal kernel. CEPA application depressed dry mass accumulation, while ethylene inhibitors improved it significantly ($P \leq 0.05$).

Water content of the kernel

The water content of the apical kernel increased sharply during the first 10 d after anthesis, and declined thereafter until maturity (Fig. 2). The application of chemicals did not change the water content of the apical kernel but, in the basal kernel, the water content increased slowly and reached a peak at 20 d post-anthesis in the control condition and declined thereafter to maturity. Treatment with ethylene inhibitors shifted the peak to d 15 post-anthesis. Application of CEPA increased the water content of the kernel during the later part of grain filling period.

Carbohydrate content of the kernel

In the apical kernel, the soluble carbohydrate content increased sharply, reaching a peak during the first 5 d of anthesis, and declining thereafter to maturity (Fig. 3). Chemical treatments did not alter the soluble carbohydrate content of the apical kernel. In the basal kernel, the soluble carbohydrate content increased slowly from anthesis, reaching a peak 15 d after, and subsequently declining. During the latter part of the grain filling period, the basal kernels possessed more soluble sugars than did the apical kernels. Application of CEPA inhibited the rate of decline in soluble sugar content of the basal kernel, whereas application of ethylene inhibitors enhanced the rate of decline. The effect of the chemical treatments on the reducing sugar content of the kernels was similar to that of the total soluble carbohydrates.

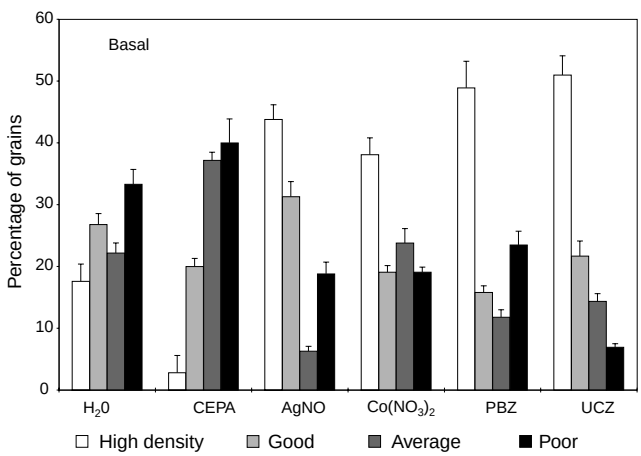

Fig. 1. The effect of ethylene action inhibitors ($AgNO_3$, $Co(NO_3)_2$, paclobutrazol, uniconazole) and an ethylene-releasing substance (CEPA) on the percentage of different grades of grains on the proximal primary branches of the rice cv. Lalat panicle. Vertical bars indicate standard deviation. Specific gravity of grains, Poor (1.0–1.06), Average (1.06–1.14), Good (1.14–1.20), High density (> 1.20).

The starch content of the kernels increased with time in a sigmoidal pattern in both apical and basal spikelets (Fig. 3). However, the rate of increase was much higher in the apical than in the basal kernels. Consequently, the apical kernel attained a higher final starch content at maturity compared to the basal kernel. The application of CEPA reduced the starch content of the basal kernel, whereas application of ethylene inhibitors improved it significantly($P \leq 0.01$). The treatment with chemicals was less effective on the apical kernels.

Activities of sucrose synthase and acid invertase in the kernel

The activities of sucrose synthase and acid invertase in the kernels increased temporally from anthesis, reaching a peak at 15 d post-anthesis, and declining thereafter to maturity (Fig. 4). Sucrose synthase activity was higher than that of acid invertase. The apical kernels possessed higher activities of both enzymes than did the basal kernels. The chemical treatments did not result in any effect on the enzyme activities of the apical kernels. In the basal kernels, application with CEPA reduced the enzyme activities, whereas they were significantly ($P \leq 0.01$) increased by treatment with ethylene inhibitors.

Experiment II

Ethylene concentration of the primary branches

The apical primary branch of the panicle possessed mostly advanced spikelets with high-density grains, and the lowermost primary branch had inferior spikelets with poor-quality grains. The release of ethylene from both apical and basal branches increased with time, reaching a peak at the time of anthesis (5 and 10 d after treatment for apical and basal branches, respectively), and declining thereafter up to

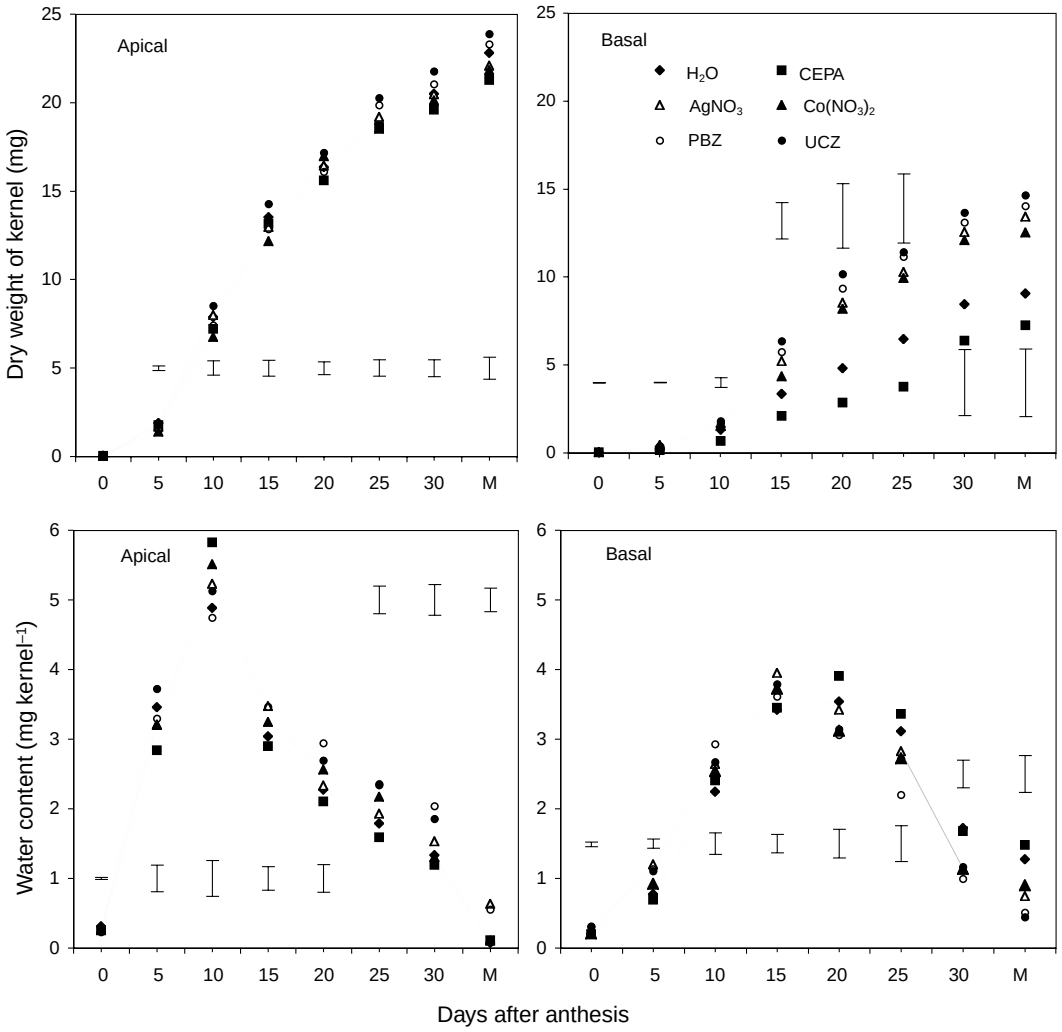

Fig. 2. The effect of ethylene action inhibitors ($AgNO_3$, $Co(NO_3)_2$, paclobutrazol, uniconazole) and an ethylene-releasing substance (CEPA) on dry weight and water content of the kernel of rice cv. Lalat. Vertical bars indicate LSD values at the 0.05 level of significance.

maturity (Fig. 5). The chemical treatments did not influence the ethylene concentration of the apical branch. In the case of the basal branch, application of CEPA enhanced the concentration of ethylene, whereas the inhibitors depressed the concentration significantly compared to the control ($P \leq 0.05$). Of the inhibitors, uniconazole was most effective in depressing ethylene concentration, whereas silver nitrate was the least effective.

Discussion

Heterogeneous development of spikelets in the rice panicle and the resulting dominance of superior spikelets of the apical branches over their proximal counterparts is not caused by any discrimination in supply of assimilates (Mohapatra *et al.* 1993). There was a need for a growth regulator signal for breaking the correlative inhibition of the basal spikelets and increasing grain yield. In the present experiment, chemicals that retarded either ethylene action or synthesis fulfilled this need. They encouraged starch synthesis as well as the activities of the enzymes responsible for cleavage of sucrose in the kernel of the inferior basal spikelets of rice panicle and increased grain yield (Fig. 1). This evidence corroborates our previous finding on the effects of such chemicals on spikelet development and grain yield of rice (Mohapatra *et al.* 2000). In contrast, application of the ethylene-releasing substance CEPA had the opposite effects on grain filling and sucrose cleaving enzyme activities in the kernels of the basal spikelets.

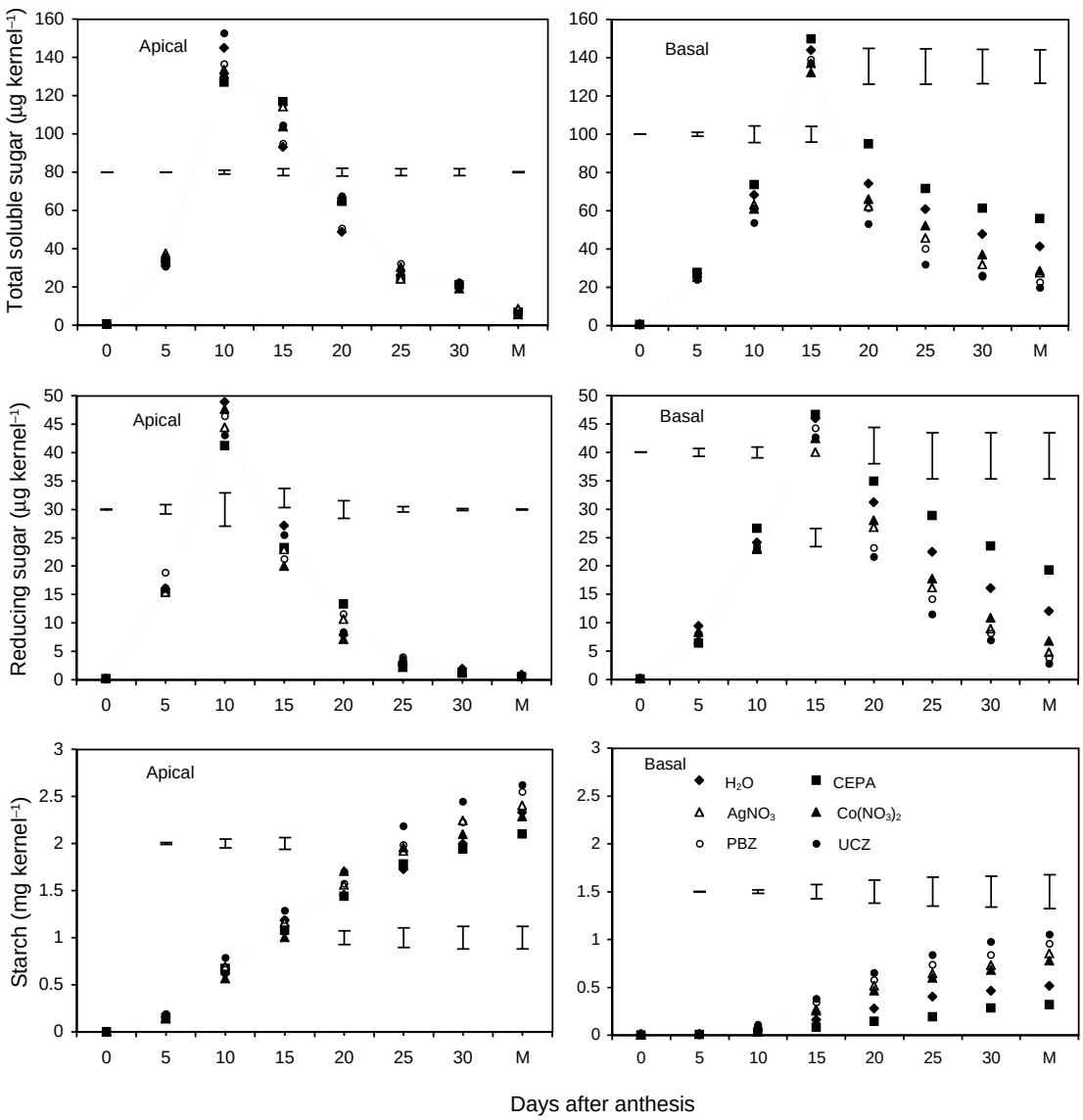

Fig. 3. The effect of ethylene action inhibitors ($AgNO_3$, $Co(NO_3)_2$, paclobutrazol, uniconazole) and an ethylene-releasing substance (CEPA) on total soluble sugar, reducing sugar and starch content of the kernels of rice cv. Lalat. Vertical bars indicate LSD values at the 0.05 level of significance.

Chemical treatment also influenced the soluble carbohydrates content of the basal kernels (Fig. 3). The soluble carbohydrate content of a plant organ measured at any particular time gives an indication of the difference between the amount received from the source and the amount consumed in growth. Assuming that the supply of sugars from the source was lower during the first half of the grain filling period than during the second, the increase in the content of sugars in the kernel during the former period is due to their slower consumption in growth and starch synthesis. The vascular connections to a plant organ become temporally more mature and organised during development, and hence, it can not be expected that the supply of assimilates to the kernel was greater in the first phase of grain development than in the second. The effect of the chemical treatments on growth and starch synthesis was not as discernible during the first phase of grain filling as that of the second (Fig. 3). Therefore, differences in the activities of sucrose cleaving enzymes and the pools of total and reducing sugar contents of the kernel during this period of grain filling were not significant, and a strong positive correlation was observed between sucrose synthase or invertase activity and sugar and starch contents of the kernel (Figs 6 and 7). This evidence suggests that activities of both enzymes were limiting starch synthesis in the rice endosperm. This inference can be supported by the observation of Chen *et al.* (1994). They found that starch synthesis in developing rice kernels was not limited by the availability of substrates, but by the activities of starch synthesising enzymes including sucrose synthase and invertase. Comparison of activity of these enzymes between the kernels of dominant apical and inferior basal positions of the rice panicle in the present experiment also supports this idea.

There are several examples where sucrose synthase has been positively correlated with starch and/or dry matter accumulation of the seed, such as maize (Prioul 1996), snap

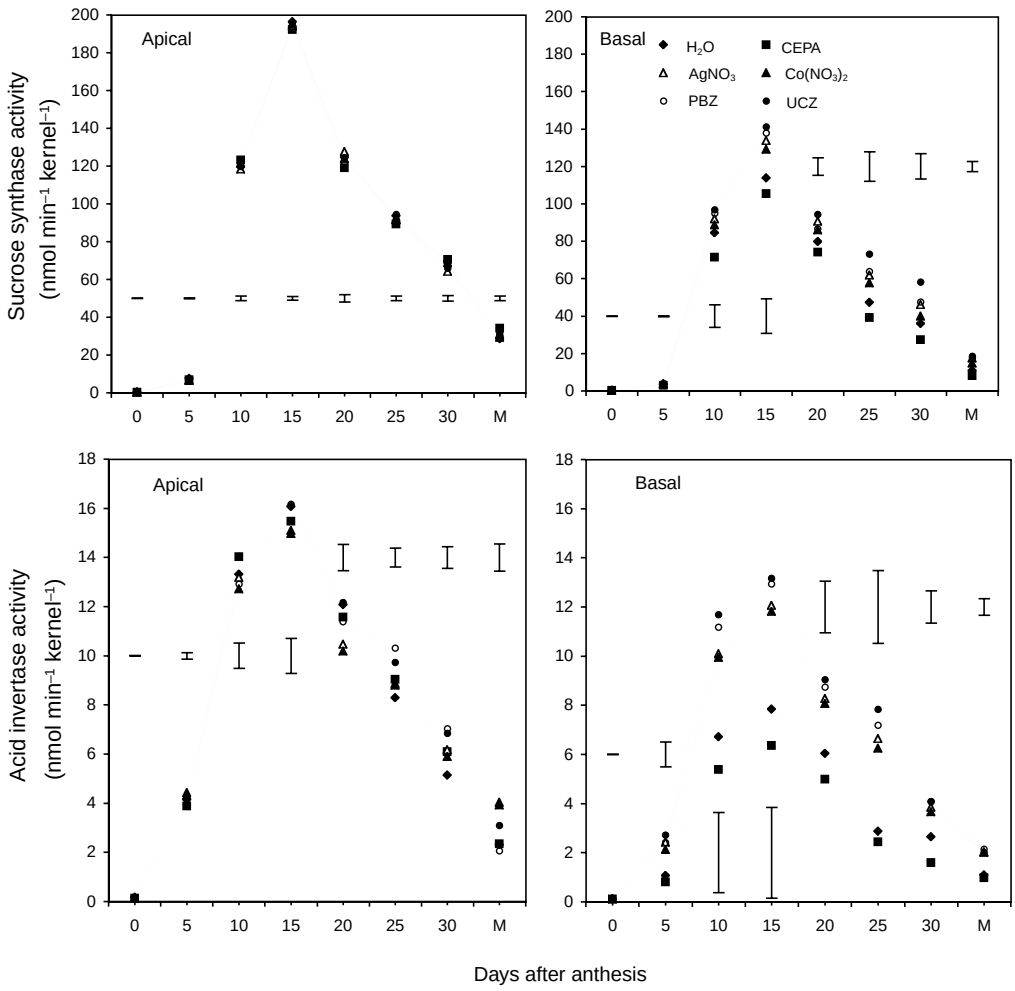

Fig. 4. The effect of ethylene action inhibitors ($AgNO_3$, $Co(NO_3)_2$, paclobutrazol, uniconazole) and an ethylene-releasing substance (CEPA) on the activities of sucrose synthase and invertase of the kernels of rice cv. Lalat during the period of grain filling. Vertical bars indicate LSD values at the 0.05 level of significance.

bean (Sung *et al.* 1994), rice (Kato 1995; Patel and Mohapatra 1996) and wheat (Dale and Housley 1986; Riffkin *et al.* 1995). However, unlike sucrose synthase, a survey of the literature does not indicate a clear correlation between soluble acid invertase and starch synthesis in the endosperm. In maize seed, both soluble and insoluble acid invertase activities regulate starch synthesis of endosperm only during the early phase of development (Xu *et al.* 1996; Zinselmeier *et al.* 1999). In contrast, the acid invertase control hypothesis proposed by Weber *et al.* (1997) for sugar import and metabolism in leguminous seeds suggests that cell wall-bound invertase is associated with the seed coat, and exhibits high activity during the pre-storage phase of seed development. Only sucrose synthase controls the entry of sucrose into the cotyledon during the storage period. Sung *et al.*(1994) reported that acid invertase activity of developing snap bean seeds was very low and did not show any significant relationship with sucrose import or growth. In tomato (Wang *et al.* 1993), the activities of both sucrose synthase and invertase were compared, and it was concluded that the former plays a dominant role in the metabolism of imported sucrose into the fruit. Similarly in wheat, sucrose synthase activity is higher than invertase activity during the entire period of seed development (Riffkin *et al.* 1995). In our experiment, although a direct positive correlation was observed between invertase activity and starch synthesis, invertase activity was much lower than sucrose synthase activity. Hence, we conclude that consumption of sucrose imported into the endosperm of rice during the first half of the grain filling period mostly occurs through the pathway initiated by sucrose synthase.

During the second half of the grain filling period in the present experiment, the sugar content as well as the activities of sucrose synthase and invertase declined progressively with time. A similar observation of temporal fluctuation of activities of sucrose synthase and invertase during the grain filling of rice kernels has been reported by Chen *et al.* (1994). Contrary to the fall of enzyme activities, the rates of absolute growth and starch synthesis became relatively higher during the second part of the grain filling period. Thus, the correlation between activities of sucrose cleaving enzymes and starch content of the kernel was negative (Figs 6 and 7). The poor correlation between the two types of parameters was due to the fact that ethylene inhibitors improved sucrose synthase and invertase activities as well as growth and starch synthesis of the kernel, whereas CEPA depressed them.

The sugar content of the kernel during the second part of the grain filling period fluctuated between the treatments, mostly according to their utilisation in growth and starch synthesis; the content was high when their demand was low and *vice versa*. Therefore, a positive correlation was observed between the enzyme activities and the sugar content of the kernel. However, the poor correlation indicates that sucrose cleaving enzyme activity (mostly sucrose synthase) was not the sole factor responsible for sink activity of the kernel during the second half of the grain filling period, although most of the sucrose entering into the starch

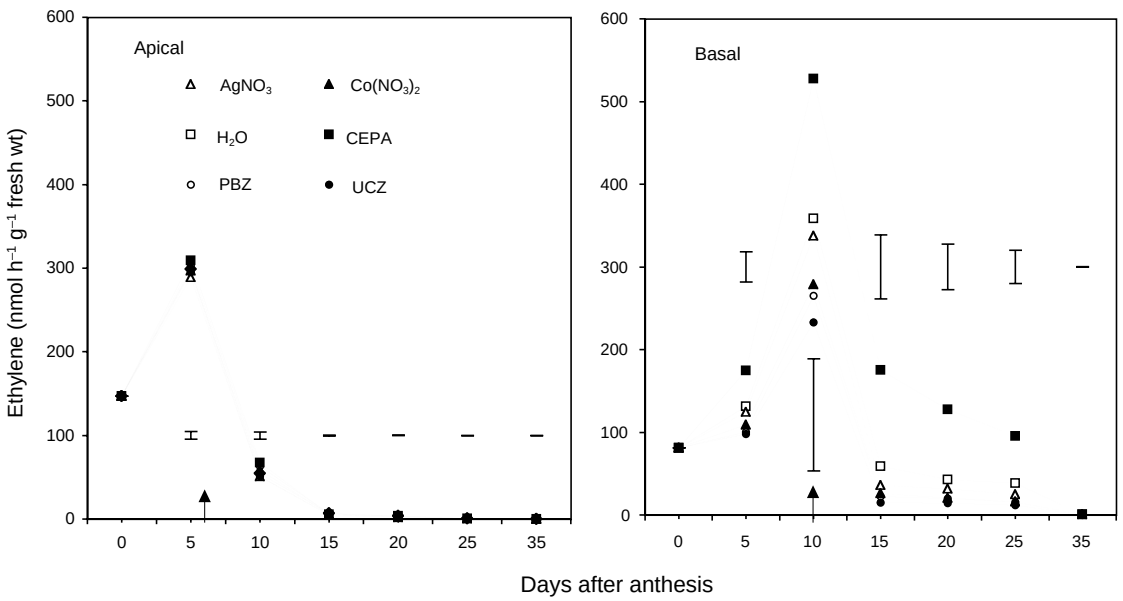

Fig. 5. The effect of ethylene action inhibitors ($AgNO_3$, $Co(NO_3)_2$, paclobutrazol, uniconazole) and an ethylene-releasing substance (CEPA) on the ethylene concentration of apical and basal primary branches of panicle of rice cv. Lalat during the period of grain filling. Vertical bars indicate LSD values at the 0.05 level of significance. Arrow indicates the time of anthesis.

biosynthesis in the endosperm prefer this enzyme for sucrolysis at the entry point (Riffkin *et al.* 1995). The role of other enzymes, such as ADPglucose pyrophosphorylase, starch synthase and starch branching enzymes, has been highlighted in the regulation of starch biosynthesis of sink tissues (Perez *et al.* 1975; Qui-Lee and Setter 1985; Chen *et al.* 1994; Umemoto *et al.* 1994; Smith *et al.* 1995, 1997). In the present situation, any one or more than one of them might be responsible for regulation of starch synthesis in the kernel of the basal spikelet of the rice panicle during the second phase of grain filling in addition to sucrose synthase.

Ethylene inhibitors stimulated the activity of sucrose synthase only in the basal kernels, and improved partitioning of materials in favour of their growth, but they were not effective on the apical kernels. The hull space between lemma and palea, which determines the size of the grain for a rice variety, is a stable genetic character (Matsushima 1970). Thus, there is no scope to accommodate any extra material and increase the dry weight of the superior apical kernel, which attains the maximum possible weight. CEPA application depressed the sucrose synthase activity of the basal kernel, but could not disturb the strong sink activity of the apical kernel. However, the strong correlative dominance of the apical kernel over the growth of the basal kernel was broken by ethylene inhibitors (Naik and Mohapatra 1999). It is reported that ethylene synthesised in one flowering organ can work as a mobile factor to induce senescence symptoms in other flower parts (Woltering *et al.* 1995). Such inter-organ

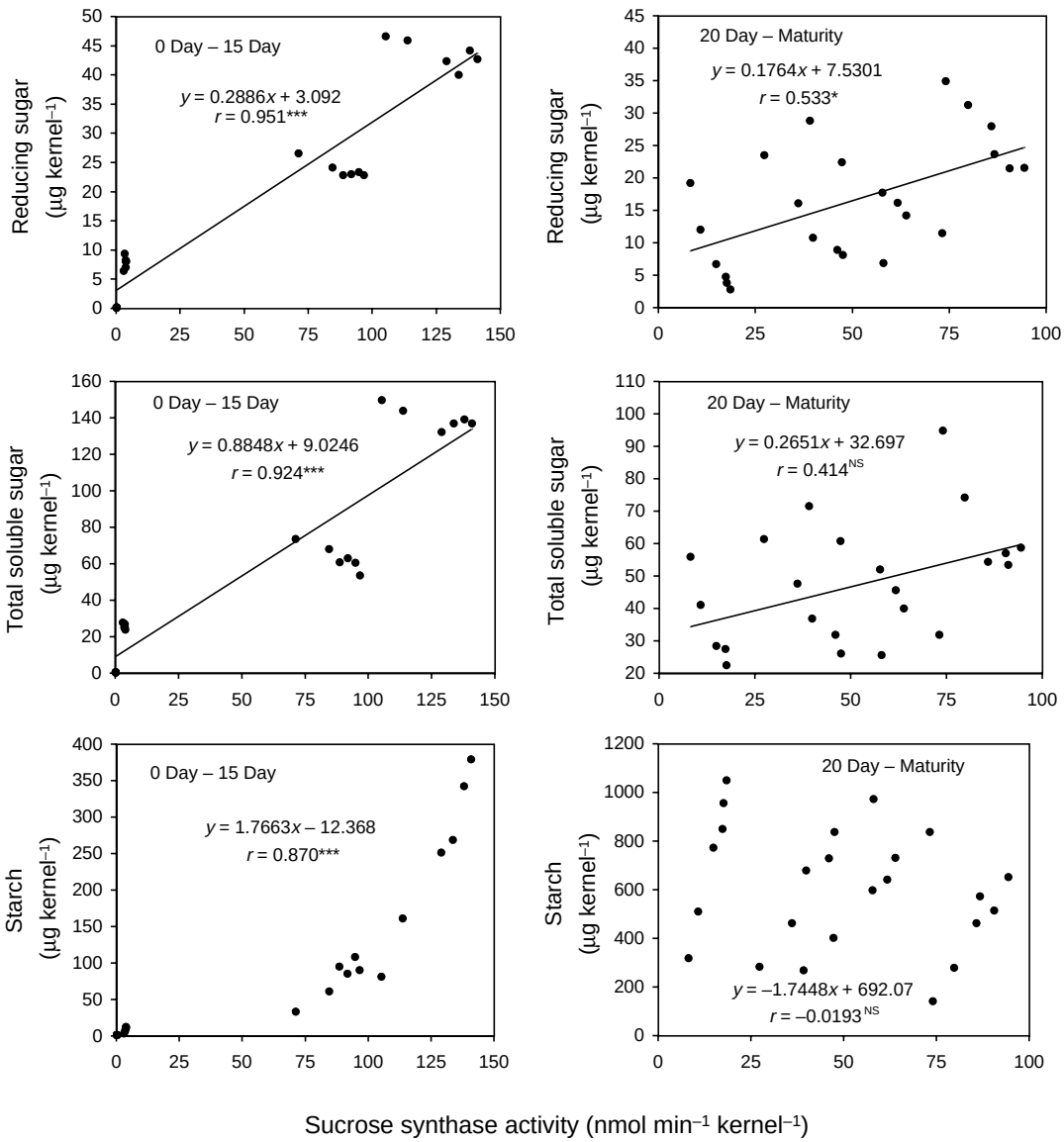

Fig. 6. Correlation between sucrose synthase activity and total soluble sugar, reducing sugar and starch contents of the basal kernel of rice cv. Lalat during the period of grain filling.

signalling between dominant apical and subordinated basal spikelets may occur in the rice panicle, mediated by ethylene.

In the present experiment, the estimation of ethylene concentration of the apical and basal primary branches (Fig. 5) supports the idea of inter-organ signalling. The concentration of ethylene was maximum at anthesis in both apical and basal primary branches. The apical branch reached anthesis 5 d earlier than the proximal branch, and hence early genesis of ethylene or its precursor from this branch might have imposed an inhibitory effect on the growth and development of the basal branch. A number of workers have reported the genesis of ethylene from the flag leaf and inflorescence of rice (Khan and Choudhuri 1992; Saka *et al.* 1992) and wheat (Labrana *et al.* 1991; Beltrano *et al.* 1994) during the period of grain filling.

In the present experiment, the chemical treatments influenced ethylene concentration of the proximal branch of the panicle more than the dominant apical branch. Early emergence from the flag leaf enclosure obviously has helped the apical branch to escape from the exposure to chemical treatments as well as detrimental effect of ethylene generated within the boot of the flag leaf sheath. Freedom from the leaf

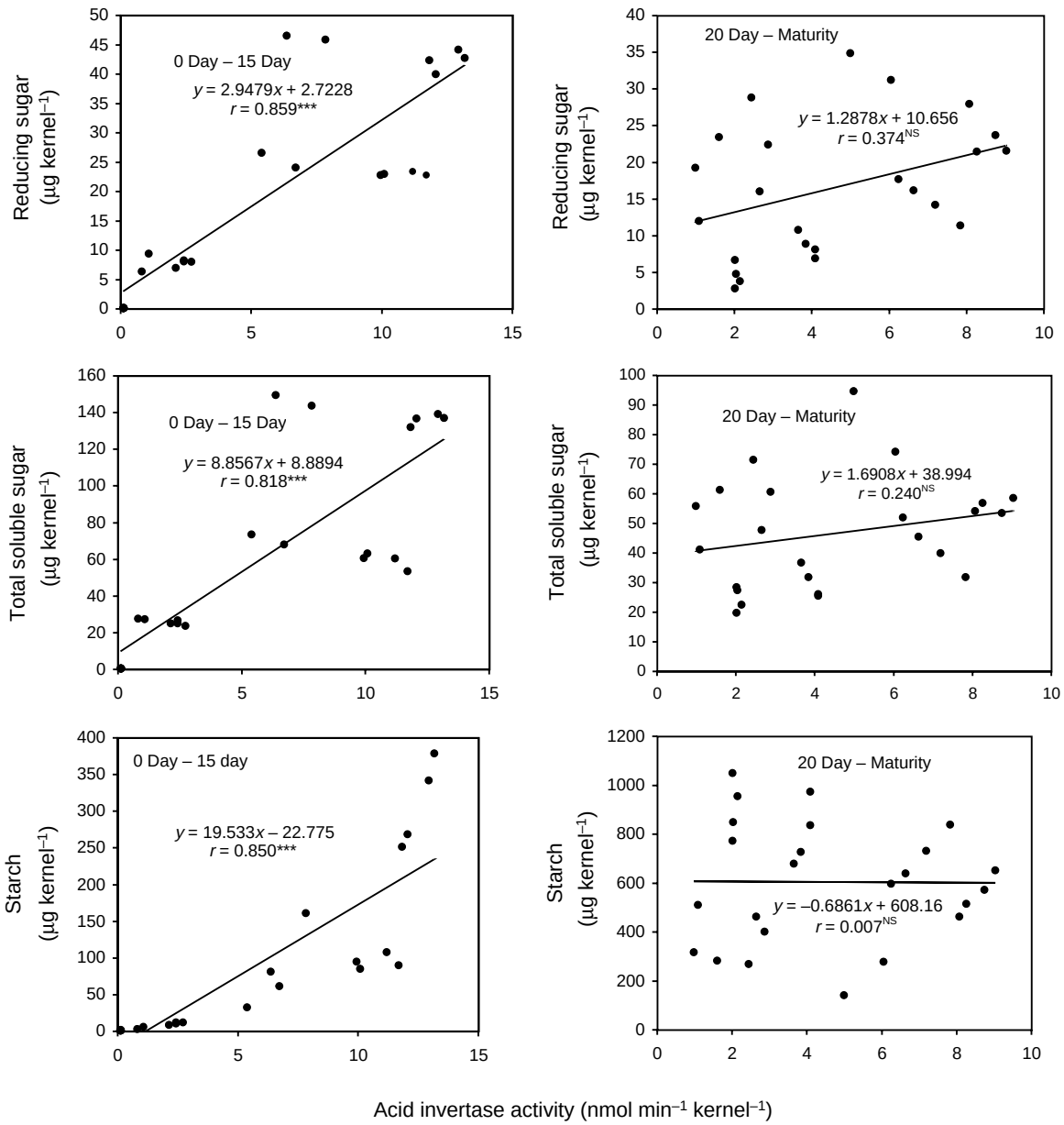

Fig. 7. Correlation between invertase activity and total soluble sugar, reducing sugar and starch contents of the basal kernel of rice cv. Lalat during the period of grain filling.

sheath enclosure also means exposure to incident light, which is inhibitory to ethylene synthesis (Gepstein and Thimann 1980; Kao and Yang 1982). The pattern of temporal fluctuation of ethylene concentration of the branches strongly supports this proposition. Because of its retention in the boot for a longer time period, the basal branch received higher doses of chemical treatment given in the present experiment. Thus, CEPA application increased the concentration of ethylene and the inhibitors depressed it. However, all the inhibitors did not exhibit identical effect in depressing the ethylene concentration of the basal branch. The ethylene synthesis inhibitors such as cobalt (Lau and Yang 1976; Lynch and Brown 1997), paclobutrazol (Wang and Steffens 1985) or uniconazole (Hofstra *et al*. 1989) reduced formation of the chemical and decreased its concentration significantly. Treatment with silver was less effective in reducing the concentration of ethylene of the basal branch than the other inhibitors, as it inhibited the action of the chemical (Beyer 1976; Philosoph-Hadas *et al*. 1994; Yu and Yang 1979), rather than formation.

The results also bring to light the stimulatory effect of ethylene inhibitors on grain filling and activity of sucrose synthase; the molecular mechanism of such stimulus is not presently understood. Ethylene-regulated gene expression has been studied with respect to senescence, fruit ripening, plant defence mechanisms (Deikman 1997) and abscission (Brown 1997). Ethylene has been implicated in regulation of the activity of hydrolysing enzymes such as cellulase, pectinase (Brown 1997) and peroxidase (Ingemarsson 1995), but nothing is known about its regulatory effect on sucrose synthase or invertase. In the cellular environment there are various physico-chemical factors, such as, pH, ion concentration, cofactors, temperature and inhibitors, which interact with enzymes and determine their activities. In case of sucrose synthase, it is known that the same enzyme catalyses both synthesis and degradation of sucrose, but factors controlling the forward and backward reactions are different (Pontis *et al.* 1981).

The influence of ethylene on these factors in relation to regulation of sucrose synthase activity is not known. It has been reported that in wheat endosperm, the activity of sucrose synthase is limited by availability of UDP and sucrose (Riffkin *et al.* 1995). In cereal seedlings, a gibberellic acid (GA) signal is believed to stimulate sugar mobilisation from the endosperm, which in turn activates the sucrose synthase gene; the resulting synthesis of the enzyme activates the breakdown of sucrose for starch synthesis of the seedling (Thomas and Rodriguez 1994). It is also reported that GA can promote transport of assimilates in the phloem (Patrick and Mulligan 1989). Ethylene is antagonistic to GA (Lieberman 1979); the inhibitors of ethylene synthesis and action can promote GA-mediated soluble sugar transport to the endosperm and induce the sucrose synthase gene for increased synthesis of the enzyme. Significant positive correlation obtained between sucrose synthase activity and sugar content of the kernel during first phase of grain filling period gives support to this hypothesis. The poor correlation observed between the two in the second phase may be a development-oriented change on the part of the kernel, where other factors (enzymes?) are involved in dry matter partitioning. The ethylene-releasing substance CEPA, on the other hand, can offset sieve tube permeability to assimilate flow by encouraging deposition of callose on the sieve plates (Thomas and Hall 1975; Fahn 1990).

Analyses of various metabolites and water contents of the kernels revealed significant positive correlation ($P \leq 0.01$) between the two parameters. The temporal fluctuations of the materials during grain development exhibited similar patterns on all occasions. The apical kernel, which had a higher final weight and better quality, also contained greater amounts of water (Fig. 2) and metabolites (Fig. 3). These observations are comparable to those for wheat (Dale and Housley 1986), and suggest that a greater amount of water mobilisation into the kernel is necessary for higher partitioning of dry matter. However, during the second phase of grain filling in the basal kernel, CEPA application increased water and metabolite contents, but partitioning was poor. A similar observation was noted in maize, where poorly developing kernels on the apical part of the panicle were found to be susceptible to 1-aminocyclopropane 1-carboxylic acid (ACC) application; the treatment reduced the rate of starch deposition and encouraged premature abortion (Cheng and Lur 1996). This evidence emphasises the need for a chemical regulator for promoting sink efficiency of these kernels. The inhibitors of ethylene action and synthesis may fulfil this role.

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