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Ethylene inhibitors improve dry matter partitioning and development of late flowering spikelets on rice panicles

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Abstract. Primary branch development of the rice panicle was in the order of a basipetal sequence from the top to the bottom at the time of anthesis. Delayed development of spikelets on the proximal branches of the panicle resulted in reduced grain filling. Two experiments were carried out to manipulate growth and development of the proximal spikelets with exogenous application of chemicals regulating formation or action of ethylene. In the first experiment, inhibitors of ethylene synthesis (cobalt) and action (silver) improved grain biomass and specific gravity of the basal spikelets, while 2-chloroethylphosphonic acid (CEPA) depressed these parameters significantly. In the second experiment, the ethylene synthesis inhibitor 1-aminoethoxyvinylglycine (AVG) promoted spikelet development on the basal primary branches and improved their survival and grain biomass. On the contrary, the ethylene precursor 1-aminocyclopropane-1- carboxylic acid (ACC) inhibited growth and development of these spikelets. The action of AVG was reversed when ACC was applied in combination with AVG. In both experiments, the chemicals did not influence growth and development of the superior spikelets on the apical primary branches of the panicle. Depression of growth and development by CEPA or ACC coincided with a concomitant rise in soluble carbohydrate concentration of the spikelets, whereas treatments with ethylene inhibitors decreased the concentration of the materials. The role of ethylene in metabolic dominance of the apical spikelets and its impact on grain yield of rice panicles is discussed.

Introduction

Most of the spikelets located on the basal primary branches of rice panicles do not produce quality grains suitable for human consumption (Padmaja Rao *et al*. 1985; Venkateswarlu *et al.* 1988). These spikelets lack demand for utilisation of assimilates and produce partially filled grains at the time of maturity (Mohapatra *et al.* 1993). The process of grain filling stops prematurely in these spikelets and the unfilled space of the hull remains occupied by water. Removal of water at maturity allows the intrusion of air into the space, and consequently, the density of the grain decreases. These grains break into pieces at the time of milling and have reduced market value. In comparison, spikelets located on the upper primary branches possess high metabolic demand for assimilates and grow faster to produce high density, good quality grains (Mohapatra *et al.* 1993).

Physiological investigations into the cause of strong metabolic dominance of the apical spikelets in development and grain filling (Xu and Vergara 1986) and the attendant correlative inhibition of basal spikelets in rice panicles discounted the possibility of any disparity in supply of assimilates in favour of inhibition of basal spikelets (Mohapatra and Sahu 1991). Application of growth regulators such as gibberellin and cytokinin improved homogeneity of spikelet development by encouraging growth and development of basal spikelets (Patel and Mohapatra 1992). The net development of a plant organ is regulated by a balance of promotive and inhibitory hormones. Thus, it may be possible that application of promotive hormones such as gibberellin and cytokinin increased the ratio between the promotive and inhibitory hormones and released the basal spikelets from the correlative dominance of the apical spikelets. However, the role of inhibitory hormones involved in such growth regulation has not been defined. It was observed that the upper three leaves (flag, second and third) of rice produce a large amount of ethylene during the period of grain filling (Debata and Murty 1983; Khan and Choudhury 1992). Saka *et al.* (1992) reported production of ethylene from the excised panicle and flag leaf blade of rice during the period of grain ripening; the latter produced more ethylene compared to the former at the time of anthesis. Similarly in wheat, the leaves and ear release ethylene during the grain filling period (Labrana *et al.* 1991) and emission from the ear is found to increase progressively from pre-anthesis to the hard dough stage of grain development (Beltrano *et al.* 1994). The lower part of the rice panicle, which remains confined to the flag leaf enclosure for a longer time than the upper part, may be subjected to the inhibitory action of ethylene more than the upper part. Hence, an attempt has been made in the present study to break correlative dominance of apical spikelets on development of proximal spikelets by application of ethylene inhibitors.

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Materials and methods

Experiment I

Plant material and experimental site

A high yielding semi dwarf rice cultivar, Swarna, was cultivated under irrigated field conditions at the Adaptive Research Station, Chakuli (latitude 21.29° N, longitude 84° E and altitude 178.8 m) during the wet season of 1997. The soil of the experimental area was a sandy loam type.

Seedlings (30 d old) were transplanted to the experimental area. The soil was ploughed and puddled before transplantation and plants were spaced at 20 × 10-cm intervals. Commercial fertilisers consisting of N, P_2O_5 and K_2O (80 : 40 : 40) were applied. Except for a few days after transplantation and before maturity, the water level in the field was maintained at 5 ± 2 cm.

Growth regulator treatments

The experimental area was divided into 4 × 3-m plots and each individual plot was considered one replicate. The plots were arranged in a randomised block design with four treatments in three replicates. $Co(NO_3)_2$, $AgNO_3$ and CEPA* were applied to the plants at the concentration of 10^{-5} M. Aqueous solution (0.5 mL) containing the chemicals was injected into the flag leaf sheath 5 d before the occurrence of anthesis of the first spikelet on the panicle. The treatment was continued for 4 d more. In the fourth treatment, the plants received only distilled water (control).

Harvesting

In each treatment, 100 plants were screened for uniform growth and development, and samples were harvested from these plants only. The time of anthesis was noted when anthers exserted from the lemma and palea of the spikelet on the tip of the uppermost primary branch of the panicle. The spikelets that reached anthesis on this day were considered as Group I spikelets. Subsequently, the event of anthesis continued for 6 d more in the panicle and accordingly, six more groups of spikelets (groups II to VII) were identified (Mohapatra *et al.* 1993). At the first sampling, two plants from each replicate plot were uprooted and the panicle on the main shoot was excised from the neck node. The group I spikelets were collected from each of the two panicles and dried in an oven at 90°C for an estimation of dry weight. On the following day, group II spikelets were collected for similar measurements. The harvesting continued for 5 d more and spikelets belonging to other five developmental groups were harvested. In the other plants spikelets positions were marked for classification into the seven developmental groups as described above. For each group, two more harvests were made, one at d 16 after anthesis and the other at maturity.

Biochemical analyses

Plant material was boiled with 80% aqueous ethanol for 30 min and the extract was transferred to a volumetric tube. The extraction was repeated for a second time and the two extracts were pooled. The volume in the tube was made up to the mark with distilled water. Aliquots of the extract were used for the estimation of total soluble carbohydrates (Yemm and Willis 1954). The residue containing no soluble sugars was utilised for the determination of starch (Buysee and Merck 1993).

Allocation ratio

The partitioning of dry matter between different groups of spikelets of the panicle was measured by method of Borrell *et al.* (1989):

$$A = \Delta W_s / \Delta W_p$$

where A = allocation ratio, ΔW_s = change of dry weight of spikelets in a group within time period *t*, and ΔW_P = change in dry weight of the panicle within the same time period *t*.

Experiment II

Plant material and cultivation

Semi dwarf high yielding rice cultivar Bhanaja (maturity duration 135 d) was used in this experiment. The plants were cultivated in the same experimental site during the wet season of 1994 in individual plots of 2 × 1.2 m^2 size in a randomised block design with five treatments and four replicates.

Chemical treatments

Plants were treated with chemicals 10 μM AVG, 10 and 100 μM ACC — (10 mM AVG + 100 mM ACC) or distilled water. All test solutions contained 0.02% Tween 20. Solution (0.5 mL) was injected carefully into the boot of the flag leaf with the help of a 1 mL syringe 5 d before anthesis of the uppermost spikelet of the panicle. The treatments were given consecutively for 3 d and discontinued thereafter.

Sampling

The first sampling was carried out on the day of growth regulator application by dissecting out the panicle from the flag leaf sheath. The panicle was cut from the plant below the neck node. The primary branches were separated from the main axis and dried in an oven at 90°C after counting the number of spikelets in each branch. Subsequent samples were taken at intervals up to the time of maturity.

Primary branch development

Branch development was quantified by summing anthesis time of the spikelets according to the method of Patel and Mohapatra (1992). The day on which the first spikelet reached anthesis in the panicle was recorded as one 'score' for that spikelet, and the score was increased by as many days as anthesis was delayed in a spikelet. When all the spikelets on a branch reached anthesis, the total developmental score for the branch was summed and averaged by dividing it with the number of spikelets on the branch. A high score indicates less development and *vice versa*.

Results

Experiment I

Dry mass and morphology of the panicle

The dry mass of the panicle increased progressively with time from anthesis to maturity in an S-shaped curve (Fig. 1). Application of ethylene inhibitors improved the dry mass of the panicle, whereas CEPA treatment reduced the weight significantly (Table 1), and the effects of the treatments were more discernible towards the later part of the grain filling period. The duration of grain filling was reduced by the application of CEPA and increased by the treatment of ethy-

*Abbreviations used: ACC, 1-aminocyclopropane-1- carboxylic acid; AVG, 1-aminoethoxyvinylglycine; CEPA, 2-chloroethylphosphonic acid; IAA, 3-indole acetic acid.

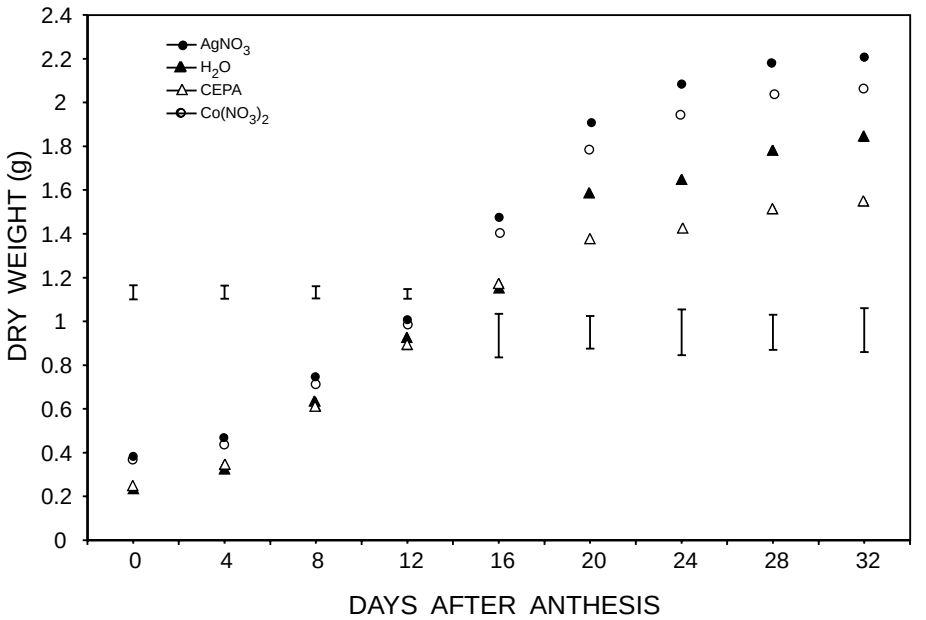

Fig. 1. The effects of ethylene synthesis [$Co(NO_3)_2$] and action ($AgNO_3$) inhibitors and ethylene-releasing substance (CEPA) on panicle dry weight of rice.

lene inhibitors by a margin of 2–3 d compared to the control. The difference in the duration of grain filling was not significant between control and chemical treatments, but was significant between chemical treatments (F-values: CEPA × $Co(NO_3)_2$ = 15.995*, CEPA × $AgNO_3$ = 19.686*). The inhibitors delayed maturity compared to the CEPA treatment. The total number of spikelets of the panicle averaged 140 and there was no significant variation between treatments. The spikelet number increased progressively from group I to IV and declined thereafter to group VII in a sequence (Fig. 2). The average weight of the grains belonging to group I spikelets was maximum and it declined in a sequence to a minimum in group VII. Group I spikelets possessed only high-density grains (specific gravity > 1.2). Grain density progressively declined from group I to VII and the latter did not have a single high-density grain. CEPA application decreased the average weight and density of the grains belonging to the last four groups significantly, whereas ethylene inhibitors improved the weight and density of these grains. As a consequence of the depression of dry weight of the spikelets belonging to the middle order groups by CEPA application, the number of spikelets increased significantly in the last two groups. On the contrary, ethylene inhibitors improved dry weight of the spikelets belonging to the last groups and thereby increased the number of spikelets of the middle order groups. The chemical treatments did not have any appreciable effect on the spikelets belonging to the early developed groups.

Partitioning of dry matter

At the time of anthesis, the allocation of dry matter was maximum in favour of group I spikelets and declined sequentially to a minimum in group VII. (Fig. 3). Between 12

Table 1. The effects of ethylene synthesis (cobalt) and action (silver) inhibitors and ethylene releasing substance (CEPA) on morphological features of the panicle in rice (cv. Swarna)

ANOVA value is significantly different from the control by using one-way and two-way ANOVA test, ^{NS} $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. A = F value between chemicals, B = F value between days (or groups), and A × B = F value between chemicals and days (or groups)

	A	B	A × B
Duration of spikelet maturity:			
$H_2O \times AgNO_3$	5.521^{NS}	—	—
$H_2O \times CEPA$	7.686^{NS}	—	—
$H_2O \times Co(NO_3)_2$	7.174^{NS}	—	—
Dry weight of panicle:			
$H_2O \times AgNO_3$	133.81***	361.99***	3.64**
$H_2O \times CEPA$	36.05***	334.56***	4.26**
$H_2O \times Co(NO_3)_2$	70.629***	358.98***	1.45^{NS}
High density grain:			
$H_2O \times AgNO_3$	104.78***	149.94***	11.62**
$H_2O \times CEPA$	1.82^{NS}	274.23***	45.30***
$H_2O \times Co(NO_3)_2$	2.51^{NS}	60.87***	8.52**
Poor density grain:			
$H_2O \times AgNO_3$	50.11***	156.25***	9.72**
$H_2O \times CEPA$	151.08***	221.43***	33.35***
$H_2O \times Co(NO_3)_2$	37.57***	182.57***	6.62**
Average weight of spikelet:			
(i) $H_2O \times AgNO_3$	14.21**	17.70***	0.41^{NS}
(ii) $H_2O \times CEPA$	8.90*	63.94***	2.05^{NS}
(iii) $H_2O \times Co(NO_3)_2$	12.59**	28.54***	0.82^{NS}
Spikelet number:			
$H_2O \times AgNO_3$	0.08^{NS}	76.90***	1.47^{NS}
$H_2O \times CEPA$	0.01^{NS}	55.05***	1.60^{NS}
$H_2O \times Co(NO_3)_2$	0.02^{NS}	59.34***	1.12^{NS}

and 16 d after anthesis, dry matter partitioning was rapid in favour of the middle order groups, while it was slow for both early and late developed groups. At maturity, the dry matter partitioning was very poor for the early developed groups as most of the materials were transported to the late developed groups. CEPA application depressed dry matter partitioning

Table 2. The effects of ethylene synthesis (cobalt) and action (silver) inhibitors and ethylene releasing substance (CEPA) on physiological parameters of the panicle in rice (cv. Swarna)

ANOVA value is significantly different from the control by using three-way ANOVA test, ^{NS} $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. A = F value between chemicals, B = F value between days, C = F value between groups, A × B = F value between chemicals and days, A × C = F value between chemicals and groups, B × C = F value between days and groups, A × B × C = F value between chemicals, days and groups

	A	B	C	A × B	A × C	B × C	A × B × C
Total soluble sugar concentration:							
$H_2O \times AgNO_3$	3.48^{NS}	149.45***	5.49***	2.26^{NS}	0.40^{NS}	6.18***	0.16^{NS}
$H_2O \times CEPA$	1.15^{NS}	114.64***	11.85***	0.34^{NS}	0.17^{NS}	10.44***	0.11^{NS}
$H_2O \times Co(NO_3)_2$	2.01^{NS}	156.32***	7.73***	1.21^{NS}	0.16^{NS}	7.80***	0.09^{NS}
Starch concentration:							
$H_2O \times AgNO_3$	14.93***	485.76***	66.24***	4.17*	0.75^{NS}	16.15***	0.16^{NS}
$H_2O \times CEPA$	4.07*	400.48***	88.90***	1.03^{NS}	0.24^{NS}	20.48***	0.07^{NS}
$H_2O \times Co(NO_3)_2$	6.85*	441.62***	69.43***	1.14^{NS}	0.30^{NS}	16.13***	0.15^{NS}
Allocation ratio:							
$H_2O \times AgNO_3$	12.96***	301.27***	62.75***	13.86***	0.65^{NS}	148.56***	1.51^{NS}
$H_2O \times CEPA$	12.13***	240.55***	41.83***	3.46^{NS}	3.02*	93.73***	0.87^{NS}
$H_2O \times Co(NO_3)_2$	15.37***	467.65***	69.82***	8.93***	0.31^{NS}	154.01***	0.83^{NS}

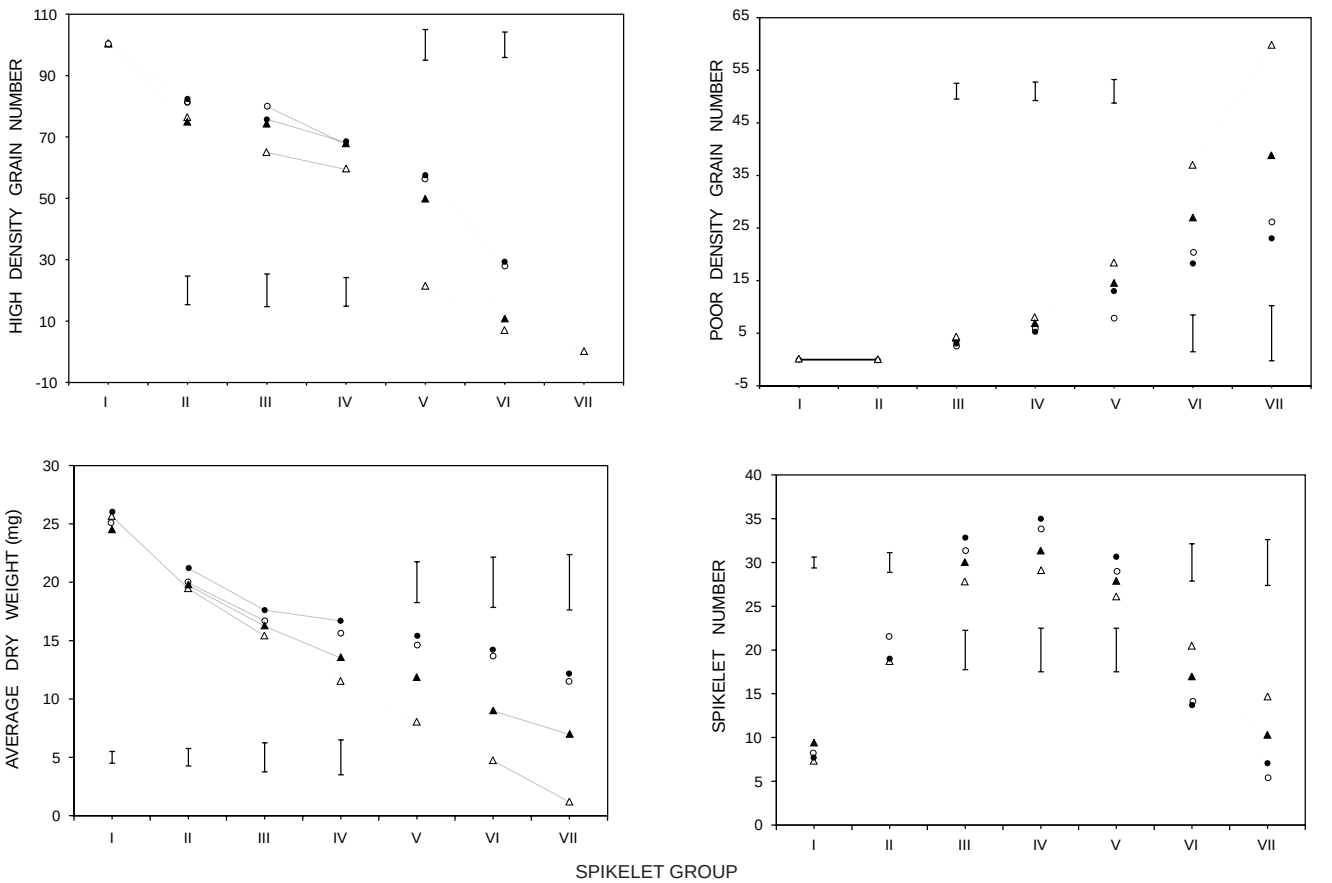

Fig. 2. The effects of ethylene synthesis [$Co(NO_3)_2$] and action ($AgNO_3$) inhibitors and ethylene-releasing substance (CEPA) on high and poor density grain number, average grain weight and spikelet number of the panicle of rice. Symbols as for Fig. 1.

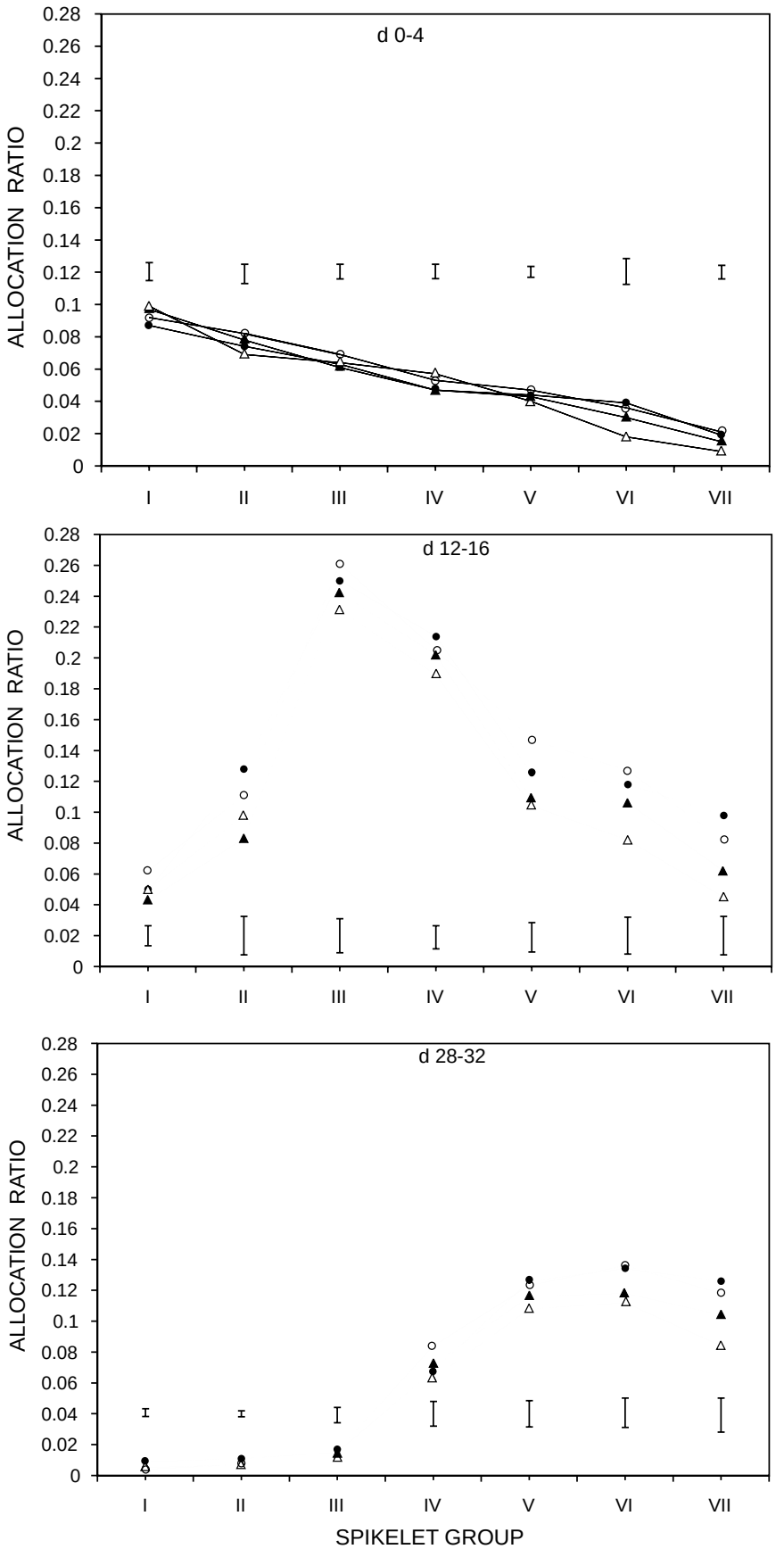

Fig. 3. The effects of ethylene synthesis [$Co(NO_3)_2$] and action ($AgNO_3$) inhibitors and ethylene-releasing substance (CEPA) on the allocation ratio of the spikelets belonging to different developmental groups of the rice panicle during the period of grain filling. Symbols are similar to Fig. 1. Group I spikelets are apical and group VII basal.

to the spikelets of the late developed groups, whereas ethylene inhibitors improved the process (Table 2). Partitioning was not affected by the chemicals in the early-formed spikelets.

Total soluble sugar concentration of the spikelets

At the time of anthesis, the total soluble sugar concentration of the spikelets was maximum in group I and declined in a sequence to group VII (Fig. 4). However, the trend was the opposite at the mid-grain filling stage and maturity, where sugar concentration was found to be higher in the late- compared to the early-formed spikelets. At anthesis stage, CEPA application lowered the concentration of sugars, whereas ethylene inhibitors improved it. However, in the other two stages of observation, application of CEPA improved the concentration of sugars and ethylene inhibitors depressed it. The effects of chemicals were more pronounced on the later- compared to the early-formed spikelets.

Starch concentration of the spikelets

The concentration of starch increased in the spikelets over time between anthesis and maturity (Fig. 5). The concentration was the highest in the group I spikelets and it declined in a sequence to group VII at all stages of observations. Application of ethylene inhibitors improved starch concentration of the late developed spikelets, whereas CEPA application depressed it significantly.

Experiment II

Growth and development of primary branches

Development was very fast in the uppermost primary branch of the panicle and it receded gradually in a sequence towards the base (Table 3). Application of ethylene inhibitors improved growth and development of the basal primary branches significantly and increased survival of more of spikelets on these branches. Consequently, the gradient in development between the apical and proximal branches was reduced. In contrast, ACC application depressed growth and development of proximal primary branches and decreased survival percentage of spikelets significantly on these branches. Application of ACC reversed the action of AVG on spikelet development when the chemicals were given in combination. Similarly to their influence on growth and development, ethylene inhibitors improved grain yield on the proximal primary branches, but had no effect on the apical branches (Fig. 6). ACC application decreased grain yield of the basal branches and improved it marginally on the distal branches. ACC application reduced average weight of the grains on the basal primary branches, whereas AVG did not have any significant effect on grain weight.

Soluble carbohydrate concentration of primary branches

The concentration of the soluble carbohydrates 5 d before anthesis was high in the apical branches and declined gradually in a basipetal sequence towards the proximal branches

Table 3. Effect of ethylene precursors and an inhibitor on (*A*) developmental score, (*B*) spikelet survival (% of max. number of spikelets), and (*C*) dry matter of primary branches of panicle in rice (cv. Bhanaja)

Values are mean for three replicates, * mean is significantly different from the control at 0.05 level using two-tailed L.S.D. test. A high development score indicates less development and *vice versa*

	Branch number								
Treatment	1	2	3	4	5	6	7	8	9
A									
Control	4.296	3.715	3.085	2.312	1.977	1.667	1.320	1.119	1.016
ACC (10^{-5} M)	4.686*	4.153*	3.393*	2.474	2.019	1.710	1.296	1.105	0.989
ACC (10^{-4} M)	4.793*	4.223*	3.511*	2.348	2.114	1.688	1.299	1.129	1.055
AVG	3.816*	3.417*	2.805*	2.210	2.011	1.695	1.299	1.150	1.050
AVG + ACC	4.350	3.817	3.105	2.371	2.015	1.703	1.295	1.097	1.080
B									
Control	60.0	64.2	76.0	91.8	90.2	98.2	96.0	97.8	97.5
ACC (10^{-5} M)	41.8*	45.7*	64.1*	83.6*	91.8	98.2	102.0	100.0	102.5
ACC (10^{-4} M)	38.2*	44.4*	61.9*	82.2*	86.9	96.4	98.0	100.0	102.5
AVG	78.2*	75.3*	84.8*	101.4*	88.5	96.4	96.0	97.8	97.5
AVG + ACC	56.4	63.0	77.2	90.4	90.2	100.2	96.0	100.0	100.0
C									
Control	155.91	248.73	324.19	319.28	265.43	268.42	248.30	236.63	211.95
ACC (10^{-5} M)	95.11*	157.33*	252.49*	281.35*	277.34	269.95	269.21	249.99	228.83*
ACC (10^{-4} M)	93.60*	150.19*	242.66*	276.77*	261.17	267.19	261.10	251.07*	227.77*
AVG	183.17*	273.95*	356.56*	349.09*	262.06	263.97	134.81	233.46	209.13
AVG + ACC	149.60	239.18	324.11	312.40	266.76	276.53	246.17	239.79	214.98

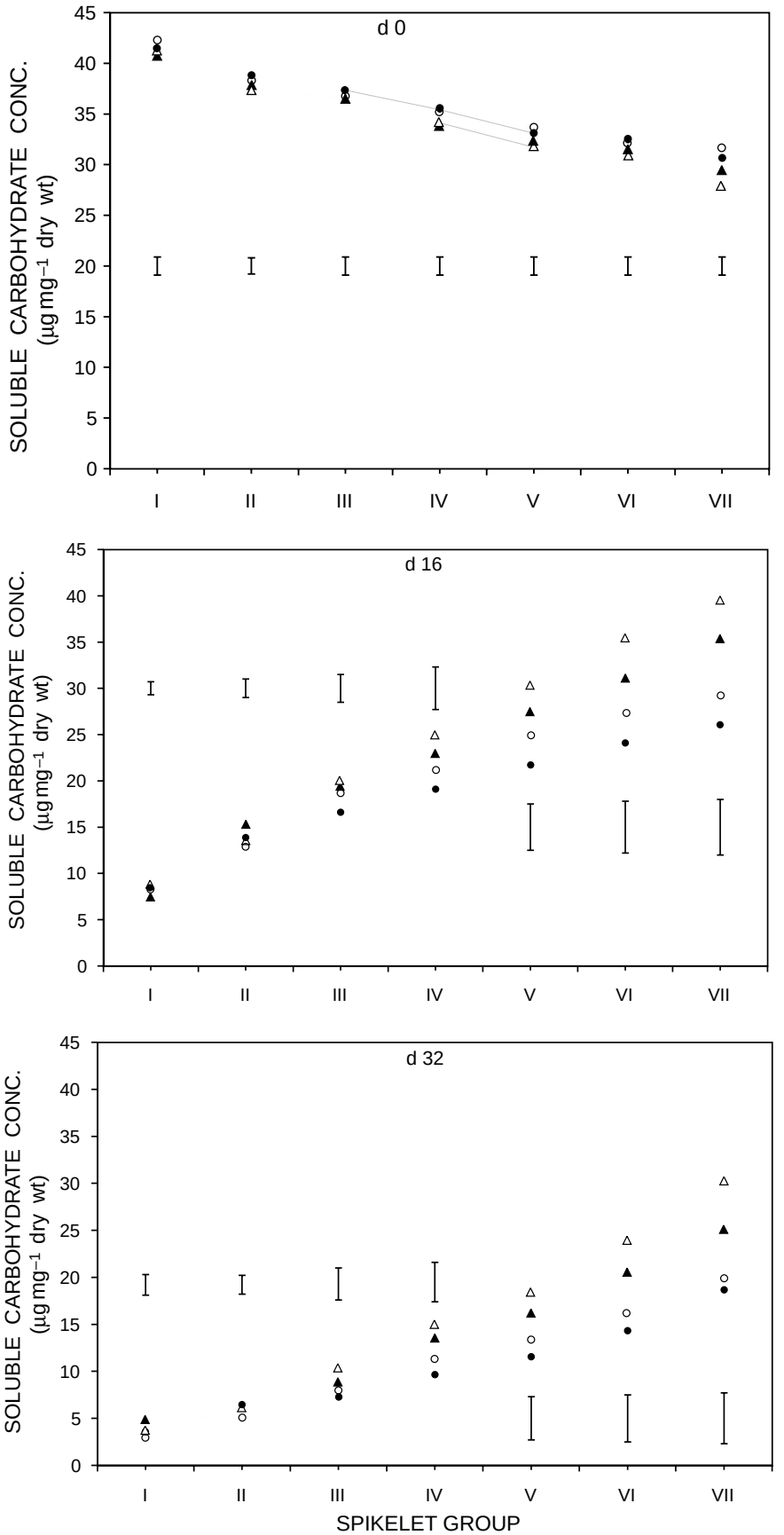

Fig. 4. The effects of ethylene synthesis [$Co(NO_3)_2$] and action ($AgNO_3$) inhibitors and ethylene-releasing substance (CEPA) on the soluble carbohydrate concentration of the spikelets belonging to different developmental groups of the rice panicle during the period of grain filling. Symbols as for Fig. 1.

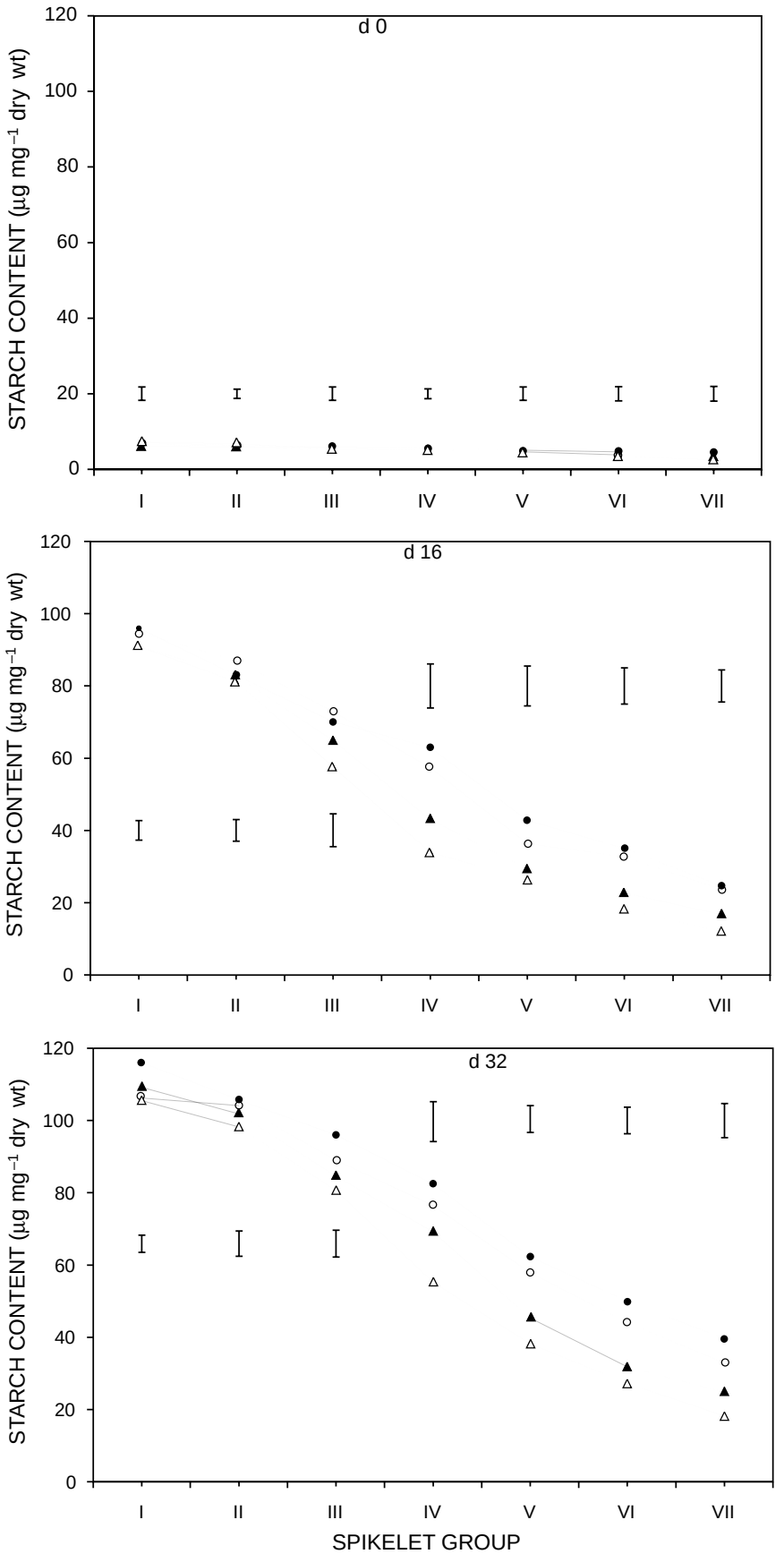

Fig. 5. The effects of ethylene synthesis [$Co(NO_3)_2$] and action ($AgNO_3$) inhibitors and ethylene releasing substance (CEPA) on the starch content of the spikelets belonging to different developmental groups of the rice panicle during the period of grain filling. Symbols as for Fig. 1.

(Fig. 7). A few days after anthesis, the sequence changed as the concentration declined in the apical branches and increased in the proximal branches. The same sequence was maintained in subsequent days of observation, although the soluble carbohydrate concentration declined temporally in all branches. Treatment of ACC increased the soluble carbohydrate concentration in the proximal primary branches, but its effect on the distal branches was not significant. Application of AVG increased the soluble carbohydrate concentration of the branches temporarily at the time of anthesis, but its effect was not significant on other days of observation.

The starch concentration of the primary branches

Starch concentration increased with time in all branches up to the time of maturity (Fig. 8). The apical branches possessed the highest concentration of starch and the concentration gradually declined towards the proximal branches in a basipetal sequence. Chemical treatment did not have any effect on the starch concentration of the middle and top branches. However, ACC treatment decreased the concentration of the lower branches, whereas AVG improved it significantly.

Discussion

Mohapatra *et al.* (1993) observed that the inferior basal spikelets of rice panicles accumulate more assimilates than they can use for starch synthesis during the process of grain filling, and ruled out a deficiency of assimilates as a causative factor for poor filling of these spikelets. Lack of competency for adequate starch synthesis may be due to lower activity of sucrose synthase (Patel and Mohapatra 1996) and/or granule bound starch synthase (Umemoto *et al.* 1994) of the endosperm. Such studies emphasised the need

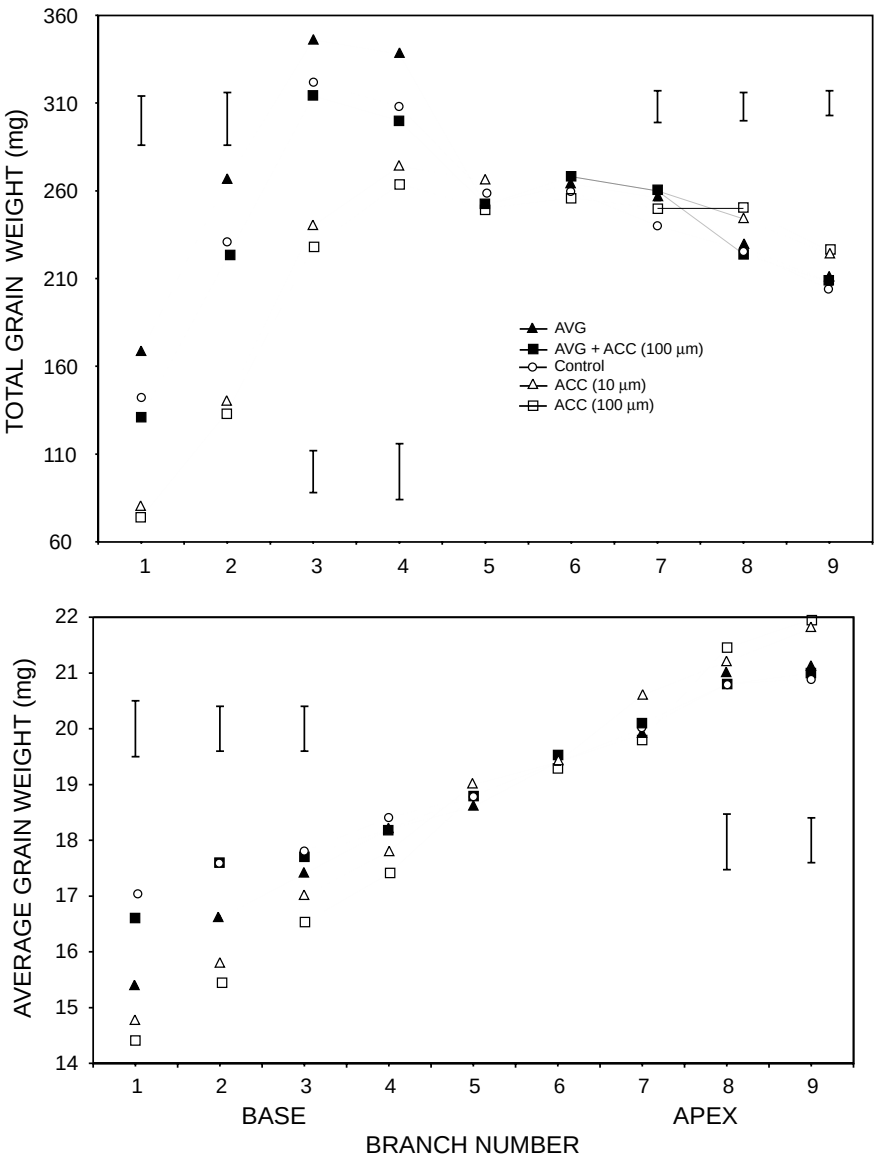

Fig. 6. The effects of ethylene inhibitor (AVG) and precursor (ACC) on grain yield of different primary branches of the rice panicle.

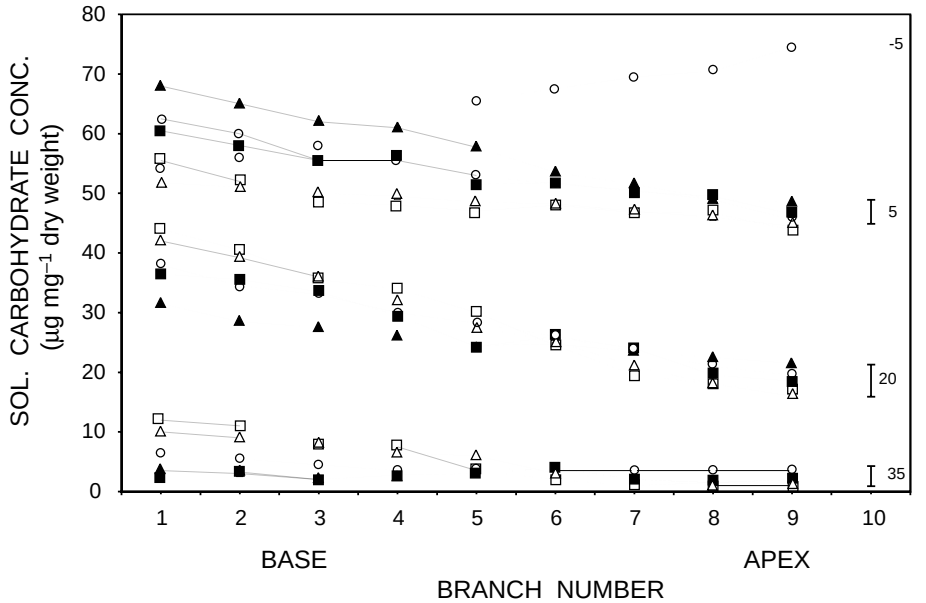

Fig. 7. The effects of ethylene inhibitor (AVG) and precursor (ACC) on the soluble carbohydrate concentration of the different primary branches of the rice panicle during the period of grain filling Numbers given adjacent to the vertical bars (LSD at 0.05), on right side of the lines, represent days from anthesis. The upward sloping line at the top represents observations taken at day 5 before anthesis (the day on which growth regulator treatments were initiated). Symbols as for Fig. 6.

for a growth stimulus that can enhance assimilate utilisation and ensure complete filling of grains. In the present experiment, application of chemicals inhibitory to ethylene action, such as silver (Beyer 1976; Philosoph-Hadas *et al.* 1994; Yu and Yang 1979), and chemicals inhibitory to ethylene synthesis, such as cobalt (Lau and Yang 1976; Lynch and Brown 1997) and AVG (Yang and Hoffman 1984; Philosoph-Hadas *et al.* 1994), have encouraged filling of poorly grain-bearing basal spikelets. In contrast, application of ethylene-releasing substances such as ACC or CEPA depressed grain quality of such spikelets further.

Since the effects of chemicals were limited to the inferior spikelets of the basal primary branches of the panicle, it may be suggested that these spikelets are susceptible to ethylene produced endogenously at the time of anthesis, and any reduction of ethylene action or synthesis can enhance grain filling. There can be several reasons for such action of the chemicals on the basal spikelet and their relative inaction on the apical spikelets. The basal spikelets remain in close contact with the flag leaf sheath for a longer period, and hence, are exposed to higher levels of endogenously-produced ethylene inside the boot (Khan and Choudhury 1992), compared to the apical spikelets. Moreover, exogenously-applied chemicals remain in the boot of the panicle bathing the basal spikelets for longer, inducing them into action. In comparison, the upper spikelets are released early from the leaf sheath enclosure and escape from exposure to intrinsically-produced ethylene or its extrinsic application. It has been proposed that ethylene produced from the dominant basal spikelets of maize at the time of pollination inhibits growth of the poorly growing kernels on the distal part of the inflorescence (Reed and Singletary 1989). Ethylene-releasing substances have been reported to cause abortion of young apical kernels of maize, while the older basal kernels are not affected (Cheng and Lur 1996). In several other plants, the event of pollination has been reported to coincide with an increase in evolution of ethylene from the stigma and style (O'Neill 1997). Thus, the poor development and partitioning of biomass in the basal spikelets of the control condition, leading to loss of grain yield, could be due to ethylene or its precursor emanating from the rapidly developing dominant apical spikelets at the time of anthesis. The findings of the present study support the proposition that ethylene-releasing substances widen the gradient in development between the apical and distal spikelets, whereas ethylene inhibitors reduce it.

Since the first dose of chemical treatment in both experiments was given near the booting stage of the plant, there was no effect on the total spikelet number of the panicle. However, ethylene inhibitors improved development of the late-formed spikelets, mostly located on the proximal primary branches, and enhanced partitioning of dry matter in their favour for grain filling. In contrast, ethylene-releasing substances acted in the opposite manner. The accumulation of soluble carbohydrates in the inferior spikelets of the proximal branches of the panicle under control conditions, and their utilisation in enhanced starch synthesis under ethylene inhibitor-treatment, emphasises the role of ethylene in the regulation of spikelet development and assimilate unloading and utilisation in the developing seed. Developing seeds are a rich source of plant hormones (Brenner 1987), but the role of these hormones in assimilate transport is unknown. Some evidence indicates that ABA encourages assimilate partitioning into developing seeds by increasing phloem unloading (King 1982; Gifford and Thorne 1986; Clifford *et al.* 1986, 1987, 1990), but this claim has been contradicted

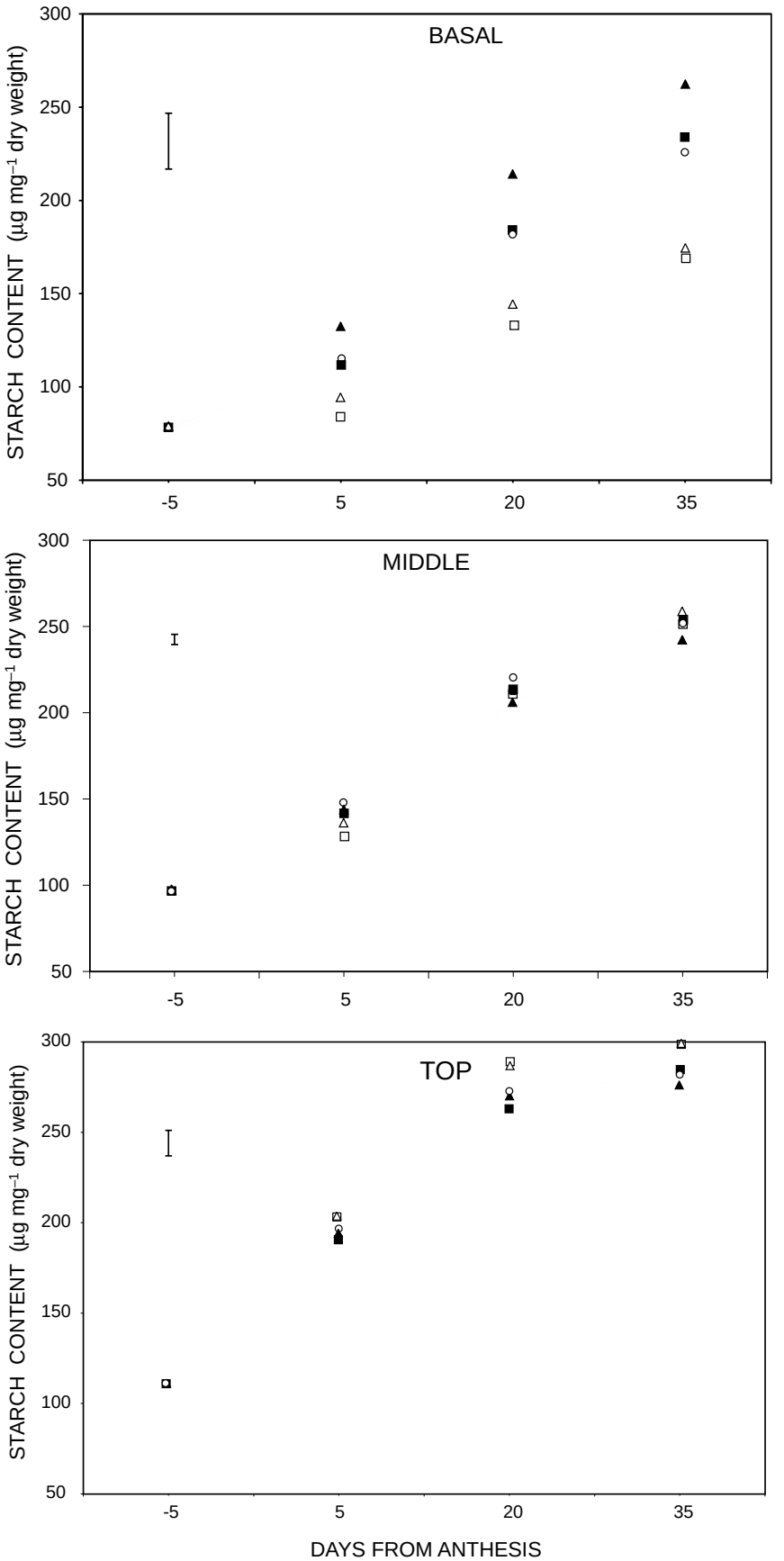

Fig. 8. The effects of ethylene inhibitor (AVG) and precursor (ACC) on the starch concentration of the different primary branches of the rice panicle during the period of grain filling. Symbols as for Fig. 6.

(Schussler *et al.* 1991, DeBruijn and Vreugdenhil 1993). Under the circumstances, it is difficult to predict the role of ethylene on assimilate partitioning into the developing seeds of rice panicles.

In order to explain the kind of correlative inhibition imposed by the early developed fruit over those developing late, Bangerth (1989) proposed the theory of primigenic dominance, where 3-indole acetic acid (IAA) transported from the latter is inhibited by the former. Depressed IAA export on the part of the subordinated fruit has been proposed to act as a signal for its reduced development. Exogenous application of IAA in rice (Patel and Mohapatra 1992) has widened the developmental gradient between apical and proximal spikelets and, in the process, has given support to the hypothesis. In the present experiments, the action of ethylene-releasing substances has mimicked the effect of IAA on rice spikelet development (Patel and Mohapatra 1992), where the inhibitors have acted in the opposite manner. These results suggest that IAA action was mediated through ethylene synthesis (cited from Moore 1989; Cline 1994).

There are several instances where application of auxin has been reported to enhance ethylene production in plants (Lieberman 1979). The action of ethylene inhibitors in improving grain setting and dry matter partitioning in the proximal spikelets is similar to the observations made earlier with hormones such as cytokinin and gibberellin (Patel and Mohapatra 1992). In barley, it was reported that the ethylene-releasing substance, ethephon, resulted in a substantial rise in ethylene release from flag leaf and spike (Foster *et al.* 1992). In wheat, ethephon hastened the process of grain maturation and senescence of the ear, whereas ethylene inhibitors such as AVG and silver thiosulphate delayed the process (Beltrano *et al.* 1994). The role of ethylene as a stimulant for grain maturation and senescence has also been emphasised by other workers (Labrana and Araus 1991; Labrana *et al.* 1991). In the present investigation, ethylene inhibitors delayed grain maturation, whereas CEPA application enhanced the process in rice panicles. The findings of the present and previous experiments (Mohapatra *et al.* 1993; Patel and Mohapatra 1996) also emphasise that low grain weight of the proximal spikelets is a result of poor sink activity rather than diminished assimilate supply. Thus, it is highly probable that ethylene may exert its effect at the cell division stage of endosperm development, but investigations linking ethylene action to cell division are lacking in the literature. It may be possible that hormones such as gibberellin and cytokinin also suppress the synthesis/action of endogenous ethylene from rice panicles during the post-anthesis period of development, and the attendant inhibition of ethylene can promote development and dry matter partitioning in favour of the subordinated proximal spikelets. In addition, the role of ethylene as a male gametocide has been emphasised in rice (Naik and Mohapatra 1999) and barley (Verma and Kumar 1978). Therefore, it is of utmost importance to ascertain the role of ethylene in the crucial processes of male and female gametophyte development, fertilisation and early stages of grain filling.

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