CHAPTER 2

CONTROLLING PLANT HEIGHT AND LODGING IN TEF (*Eragrostis tef,* Zucc.) USING GIBBERELLIN BIOSYNTHESIS INHIBITORS

2.1 Abstract

Tef (*E. tef*) is a small seeded nutritious cereal and a primary food source in Ethiopia grown on over 2.56 million ha in Ethiopia. Tef productivity is low, 1.0 t per ha, due to several factors, among which lodging is the most critical causing direct losses of about 23% under natural condition. High yielding cultivars are usually tall and more susceptible to lodging and breeding effort has not yet succeeded to decouple height from yield. Inhibitors of gibberellin (GA) biosynthesis such as chlormequat chloride (CCC) are used extensively to restrict growth and improve lodging resistance in cereals. First, responsiveness of tef plants to GA_3 and CCC application was determined using two tef varieties Gea Lammie (short) and DZ-01-196 (tall). At 10^{-2}M CCC plant height was reduced by 43% and 21% in the tall and short variety, respectively, within six weeks after plant emergence. CCC at 10^{-1}M reduced tiller number in both varieties. More detailed analysis of growth regulator application by including Paclobutrazol (PBZ) on the tall tef variety DZ-01-196 revealed that, both CCC and PBZ reduced culm length, with a much stronger reduction from paclobutrazol. Grain yield on the other hand was not affected by CCC treatment. CCC-treatment reduced culm length by affecting all internodes, with the 1st- 3rd internodes, followed by the 6th and 7th most severely affected, whereas paclobutrazol treatment strongly affected all internodes, with greatest effect on the uppermost 4 internodes. Internode diameter was unaffected by both CCC- and paclobutrazol-treatments. A steady increase in mean internode diameter until the 6th internode was found for CCC-treated and also control plants revealing a poor tapering in tef plants. Reduction of GA amount in tef might be a target for improving lodging resistance allowing uncoupling of plant height and yield.

2.2 Introduction

Tef (*Eragrostis tef* (Zuccagni) Trotter) is a panicle bearing, small-seeded nutritious cereal grown extensively in Ethiopia in diverse climatic and soil conditions with low risk of failure (Assefa *et al.,* 2010). Tef is grown on about 2.6 million ha and accounts annually for about 28% of the total acreage of cereal production in Ethiopia. However, tef suffers from low productivity with average yields of only 1.0 t ha^{-1}. Among the factors contributing to low yield, lodging is the most important (Assefa *et al.,* 2010; Tefera *et al.*, 2003; Yu *et al.,* 2007).

In general, lodging interferes with water and nutrient transport, reduces light interception, provides a favourable environment for disease, increases harvesting cost and losses and decreases grain yield and quality (Tripathi *et al*., 2003). It occurs either by buckling / bending at the basal culm internodes, or due to root lodging or failure of the anchorage system of the plant (Assefa *et al*., 2000; Ketema, 1983; Pinthus, 1973). Culm length and the strength of the basal part of the culm are considered major factors associated with lodging sensitivity (Rajala, 2003; Tripathi *et al*., 2003).

In cereals, improvement of lodging resistance has been predominantly achieved by reducing plant height, in particular by chemical inhibition of gibberellin (GA) production (Rademacher, 2000) or by the use of semi-dwarf varieties with reduced GA biosynthesis or signal transduction (Hedden, 2003). Chlormequat chloride (CCC), the most commonly used plant growth retardant (PGR), blocks GA biosynthesis by inhibition of the cyclization of geranylgeranyl diphosphate (GGPP) to *ent*-copalyl diphosphate (CPP) by CPP synthase (Rademacher, 2000). Triazole PGRs, such as paclobutrazol (PBZ), inhibit the conversion of the GA precursor *ent*-kaurene to *ent*-kaurenoic acid (Rajala, 2003; Hedden and Graebe,

1985). In general, PGRs have been extensively used in many crops to reduce lodging through shortening of the stem and to maintain a steady improvement in grain yield (Berry *et al.*, 2004; Rajala, 2003).

Reduction in plant height due to PGR treatment, is associated with reduced elongation of internodes particularly of the uppermost internodes and peduncle (Sanvicente *et al*., 1999; Rajala, 2003). CCC inhibits stem elongation in wheat (Humbries *et al.*, 1965) and in oilseed rape. Foliar treatment with a combination of CCC, ethephon and imazaquin reduced main stem length in barley where shortening of the uppermost three internodes contributed significantly to the reduction (Sanvicente *et al.*, 1999). PBZ application was found to reduce stem length and lodging in rice and increase yield by up to 15% compared to controls (French *et al*., 1990).

In tef, cultivars bred for improved grain yield possess a tall phenotype and are highly susceptible to lodging (Assefa *et al.,* 2010; Yu *et al*., 2007). Thus, lodging susceptibility has prevented the introduction of higher yielding varieties with good grain quality, and also hampered the use of input-intensive husbandry. Currently, there is no detailed information available for tef on the effect of PGR treatment on lodging and yield responses. The objective of this study was therefore to investigate morphological and yield changes in tef following GA biosynthesis inhibitor treatment under controlled environmental conditions. Results obtained demonstrate that CCC treatment of tef plants significantly reduces plant height without affecting yield.

2.3 Materials and Methods

Two experiments one with preliminary observation (also referred in this chapter as Experiment I) involving two growth regulators and two varieties and a second one (also referred to as Experiment II) involving one variety and two growth regulators have been carried out.

2.3.1 Plant material

Seed material of the tef (*Eragrostis tef*) varieties DZ-01-196 and Gea Lammie used for the experiments was obtained from the Ethiopian Institute of Agricultural Research, Holetta Agricultural Research Center, Ethiopia. Plants of variety DZ-01-196 have a tall phenotype, derived from a conventional breeding program and is widely used for cultivation while Gea Lammie is a landrace grown by farmers for its earliness.

2.3.2 Plant growth

Experiment I was done using varieties DZ-01-196 and Gea Lammie grown in a greenhouse at University of Pretoria. Plants were grown on a germination soil mix in a pot supplemented with a Hoagland fertilizer solution (Kebede *et al.,* 2008). With the second set of experiments, plants were grown at Rothamsted Research, UK from May to August using pre-germinated seeds in moist soil in pots. In both experiments a total of 12-16 plants were maintained in 3 to 4 per treatment. About 7-10 days after emergence thinning was done or transplanting of uniform seedlings was carried out to new pots [15 cm diameter (top) x 12.5 cm (height) and 10 cm diameter (bottom)]. Seedlings were grown on either a commercial germination mix

soil supplemented with half strength Hoagland solution in the observation trial or in a compost mix consisting of peat (75%), sterilized loam (12%), vermiculite (3%) and grit (10%), which was supplemented with a slow release fertilizer. Plants were well-watered every other day and the temperature was maintained at 23-27°C (day) and 15-18°C (night). Seedlings were grown for 14 weeks until plant maturity in an environmentally controlled greenhouse using a 16-h photoperiod provided by natural light supplemented with light from sodium lamps to maintain a minimum PAR of 350 µmol m^{-2} s^{-1}.

2.3.3 PGRs treatment

In the first experiment, the above two genotypes were considered for the investigation. Gibberellic acid (GA_3) and a GA-biosynthesis inhibitor, chlormequate chloride (2-chloroethyl-N,N,N-trimethyl-ammonium chloride, CCC) were applied by rubbing the underside of the leaf lamina. Application began after plant thinning and continued for six weeks every week. Both compounds were applied (10µl) to the lower surface of the uppermost expanding young leaf per week, at concentrations ranging from 10^{-1}M to 10^{-6}M. In the second experiment, only one variety, DZ-01-196 was considered for a more detailed study. Plants were treated with CCC and a potent GA- biosynthesis inhibitor, paclobutrazol (PBZ), (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol). CCC was applied at 10 mM and 100 mM and PBZ at 10 µM and 100 µM. For both inhibitors, individual solutions were prepared in dsH_2O and 100 ml of individual solution were applied every two weeks to the base of the pot when watering of plants was carried out. Treatment with inhibitors started 3 wks after seed germination.

2.3.4 Growth measurements

Culm, individual internodes and panicle lengths of tillers were measured from the subtending nodes using a ruler. The internode and tiller number were also recorded for each plant. Primary tiller refers to the main tiller that emerges first as the seed germinates. Secondary tillers refer to shoots that emerge at a later stage during seedling growth. The internode diameter was measured at 3 mm above the node using a Standard Digital Caliper. Dry weight was determined from above-ground plant material by drying fresh material at 80^{o}C for 2 days in an oven. Grain yield was determined by measuring the weight of seeds from all tillers.

2.3.5 Analysis of endogenous GA content

The upper most two internodes including their nodes at shoot elongation stages and just before panicle initiation were harvested and stored at -80^{0}C until analysis. Endogenous GA levels were monitored using stored internodal tissue after samples were freeze-dried and grinded using a ball mill for extraction, purification, and analysis of GAs. Powdered replicate samples of about 0.5g were re-suspended in 80% aqueous MeOH with addition of mixture of 2H- and 3H-labeled GA internal standards. The aqueous extract was then subjected to a rotation vacuum evaporator at 40-45^{0}C to remove methanol. The pH of the aqueous extract was adjusted to 3.0 using 1 mol/l HCl before further partitioning three-times with water-saturated ethyl acetate. The combined organic phases were reduced to dryness under vacuum at 42^{0}C to remove ethyl acetate. After column purification and full methylation with ethereal diazomethane, samples dissolved in methanol were injected onto an analytical C18 reversed phase HPLC column for fractionation. Recovery of fractions was monitored using triturated

(3H) internal standards and GAs were quantified using gas chromatography- mass spectrometry (GC-MS) system using selective ion monitoring.

2.3.6 Data analysis

Growth and yield data were collected after six weeks and at plant maturity for the first and second sets of the experiments from 12 individual plants and their tillers per treatment. Analysis of variance (ANOVA) and Pearson Correlation Coefficients were performed for data analysis using the SAS statistical package (SAS Institute Inc., Cary, NC, USA). Statistical significance of difference between treatment means was determined using the Tukey's Studentized Range (HSD) Test. A *P*-value of <0.05 was considered as significant.

2.4 Results

2.4.1 Experiment I

The effect of exogenous application of gibberellic acid (GA_3) and the GA inhibitor (2-chloroethyl-N, N, N-trimethyl-ammonium chloride (CCC) on two tef varieties, Gea Lammie and DZ-01-196, was investigated. In this preliminary observation, the two genotypes showed, to a considerable degree, contrasting response to the application of exogenous GA_3 and CCC. Different GA_3 or CCC amounts affected plant height beginning in the first week of its application in both varieties when compared to untreated control plants. In general, GA_3 application increased plant height whereas CCC reduced the plant height in both varieties (Fig. 2.1A and B).

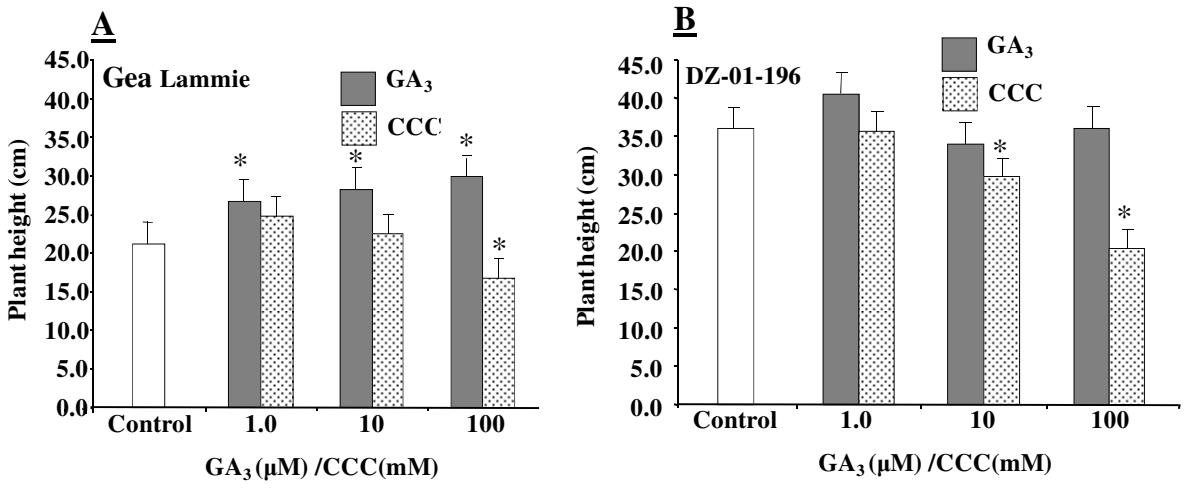

Figure. 2.1A and B. Growth (plant ht) response after six weeks of Gea Lammie and DZ-01-196 grown in greenhouse to exogenous application of GA and CCC. Treatments: C=Control; GA_3/CCC: 1= 1µM/1mM, 10=10µM/10mM and 100=100µM/100mM

Application of GA_3 (10^{-2}M) significantly increased ($p<0.01$) plant height by 41% in plants of the short genotype and 13% of the tall genotype under greenhouse conditions. In contrast, applying CCC (10^{-2}M) significantly reduced ($p<0.01$) plant height by 46% in the tall genotype compared to a 27% reduction in the short genotype. GA_3 treated plants were tall and had slender stems compared with plants treated with CCC (data not shown). The above results led to further study in more detailed (second experiment) only one variety, DZ-01-196, using CCC and PBZ. Results show that both fresh and dry weight of DZ-01-196 plants were affected by CCC treatment. At a CCC concentration of (10^{-1}M) fresh weight of plants was significantly reduced ($p<0.001$) in the taller genotype when compared to the untreated control (Fig. 2.3). Number of emerging tillers was also significantly ($p<0.05$) reduced by the highest CCC level (10^{-1}M) in the taller genotype. There was at most one tiller per plant at elongation stage (in addition to the main tiller) in those treated with CCC ($\geq 10^{-1}$M) when compared to the untreated control (Fig. 2.2).

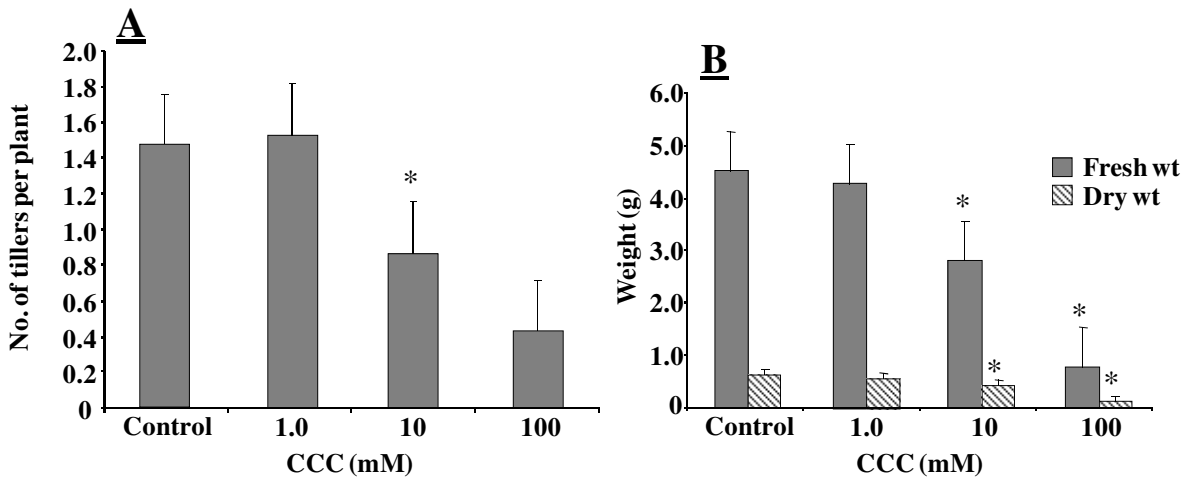

Figure 2.2. Tillering (A), fresh and dry weight (B) responses of six weeks old DZ- 01-196 seedlings to CCC treatment.

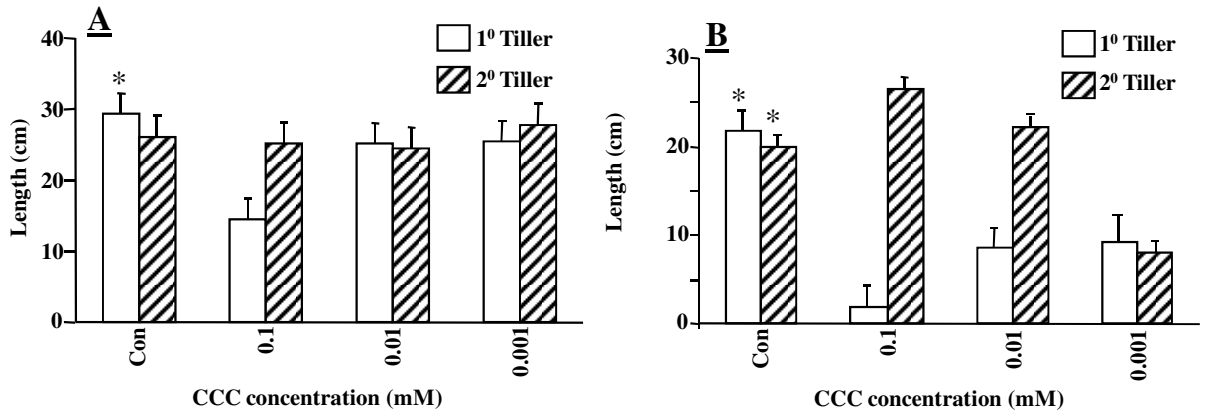

Figure 2.3 Effect of foliar applied CCC at different concentrations of foliar applied CCC on the length of the 7th internode (A) and 8th internode (B) of primary (1^{0}) and secondary (2^{0}) tillers in comparison to the untreated control. Data represent the mean ± SE of tiller length of 12 individual plants.

Most of the reduction in the culm length was due to a significant reduction in internode elongation particularly in the upper-most two internodes following CCC treatment (Fig. 2.3 A and B). However, in the uppermost (8th) inernode, it has been observed that the primary tiller has been more sensitive to CCC and the secondary tiller. Length of the secondary tillers was reduced at lower CCC levels than at higher level compared to the main tiller. Such contrasting response was not observed between the main and secondary tillers for the 7th internode. The reason for this is not clear.

2.4.1.1 *Analysis of endogenous GA content*

The two genotypes of tef, Gea Lammie and DZ-01-196 were characterized to determine the state of GA concentrations in them and reveal the existence and nature of the relationship between GA and plant height.

The predominant GA pathway and the primary biologically active product in vegetative shoot of tef was previously unknown. On the other hand, endogenous GA content determination in plant material is a challenging task because of GA's extremely low concentration in plants and its complex biological matrices (Ge *et al*., 2007). Other characteristics such factors as low ultraviolet (UV) absorption, absence of fluorescence, and distinguishing chemical characteristics as well as the need for specific chemical assay makes GA analysis complicated (Ge *et al*., 2007). Therefore, the determination of GA levels in plants had to be carried out at Rothamsted Research/UK where expertise and facility were found.

Results from the endogenous GA analyses of plant tissues taken from the upper most two internodes including their nodes using analytical HPLC column fractionation and GC-MS monitoring shows that the shorter genotype Gea Lammie has generally lower level of bioactive GA and most of the precursors than the tall genotype (Table 2.1). The content of the most abundant bioactive GA_3 was about three fold less in the shorter genotype Gea Lammie compared to DZ-196-01. The bioactive GA level corresponds with plant heights, and associated other growth characteristics of the two genotypes. Moreover, the concentration of most of the immediate precursors in Gea Lammie were about half the amount in DZ-01-196. The amount of endogenous GA_1 concentration was moderately related with plant height than biomass or number of tillers. Concentrations of precursors for the Non- 13-Hydroxylation pathway such as GA_{15}, GA_9, and GA_{34} were extremely low or nil in most cases. The amount of and the bioactive final product, GA_4, of this pathway was nil in both genotypes (Table 2.1). Therefore, the analysis showed that the early 13-hydroxylation is the major or preferred pathway in *E. tef* and the most abundant bioactive form was GA_1 (Table 2.1).

Table 2.1 Quantification of GA intermediates and bioactive forms from internode sample analysis at stem elongation stages of two tef varieties, DZ-01-196 (tall) and Gea Lammie (short).

Genotype	**GA_1**[§]	**GA_{29}**	**GA_3**[§]	**GA_{15}**	**GA_4**[§]	**GA_8**	**GA_{20}**	**GA_{19}**	**GA_{34}**	**GA_9**	**GA_{53}**
DZ-01-196	5.71[**]	2.25	3.17	0.0	0.0	9.36	6.65	19.22	0.18	ND	10.68
Gea Lammie	4.22	1.12	1.07	0.0	0.0	5.35	4.63	10.97	0.3	0	4.28
SE	0.25	0.71	1.39			0.53	0.31	0.53	0.28	1.06	0.25

ND =not determined; [§]Bioactive forms of GA; [*]*E. tef* has the 13β-hydroxylation as a major pathway for GA biosynthesis hence GA_1 is a major bioactive product.

[**]Values are in ng per g dry weight and a data point represents average of three samples

2.4.2 Experiment II

2.4.2.1 *Culm and panicle length*

In the second experiment treatment of tef plants with PBZ using soil application of the growth regulator significantly reduced culm and panicle length of plants by internode shortening when compared to CCC treatment or to the untreated control (Fig. 2.4; Table 2.2). PBZ also showed significant effects on growth parameters at a much lower concentration than CCC (Table 2.1). When plants were treated with PBZ at 10 µM and 100 µM, culm length was reduced by 92% and 98%, respectively. However, CCC treatment only reduced

culm length by 9.3% (10 mM) and 22.3% (100 mM). Further, PBZ treatment significantly reduced also panicle length (Table 2.2). In contrast to PBZ treatment, CCC treatment increased panicle length at both concentrations applied (10 mM and 100 mM) with a significant increase at 100 mM when compared to the untreated control (Table 2.2). In addition, the panicle length to culm length ratio of 1.61 (10 µM PBZ) and 1.51 (100 µM PBZ) decreased to 0.37 and 0.49 when plants were treated with either 10 mM CCC or 100 mM CCC, respectively. This indicates that both inhibitor treatments had a stronger effect on culm length than on panicle length (Fig. 2.5), with CCC treatment resulting in an increased panicle length at both concentrations. Furthermore, after PBZ and CCC treatment panicle length was strongly correlated with culm length, culm dry weight and total above ground shoot dry weight and also negatively correlated with tiller number per plant and dry weight per height ratio (Table 2.5).

2.4.2.2 *Internode growth*

Both internode length and diameter were significantly reduced by PBZ treatment as a soil application when compared to CCC treatment or to the untreated control (Tables 2.2 and 2.3). In PBZ-treated plants, internode length was reduced in all internodes. However, the two upper most internodes of plants treated with 10 µM PBZ and the four uppermost internodes of plants treated with 100 µM PBZ completely failed to elongate (Table 2.3). In CCC-treated plants, reduction in internode length also varied between the different internodes. The first three internodes (internodes 1-3) contributed 48%, whereas the last three internodes (internodes 5-7) contributed 46% to the total internode length reduction. In the preliminary study when CCC was used as a foliar application and not as a soil application, the upper-most two internodes contributed most of reduction in internode length (See Fig. 2.3: A and B).

However, for the 8th internode, length of the secondary tillers was less at lower CCC levels than at higher level.

The lowermost two internodes (I1+I2) of CCC-treated plants were further more positively correlated ($r = 0.70$, $P < 0.05$) to culm length when compared to the uppermost two internodes (I7+I8) ($r = -0.04$, $P < 0.05$) (data not shown).

Table 2.2 Effect of CCC and PBZ on culm and panicle length, number of tillers and seed weight per plant of tef cv. DZ-01-196.

Treatment	Culm length (cm)	Panicle length (cm)	No. of Tillers	Seed wt (g)
Control	159.9± 3.7a	50.26±1.9c	5.0±0.6b	3.79±0.5a
CCC				
10mM	145.0±2.2b	53.93±0.9b	6.0±0.6b	3.63±0.7a
100mM	124.2±3.3c	61.50±0.7a	7.3±1.1b	3.78±0.3a
PBZ				
10µM	11.5±1.1d	18.57±0.8d	17.3±2.8a	1.11±0.3b
100µM	3.9±0.3e	5.87±0.3e	15.0±2.1a	0.05±0.03b
Significance	***	***	***	***

Letters within the column denote significance as determined using the Student's *t*-test. Data shown represent mean values ±SE of 12 individual plants. Significance level was determined using ANOVA (*** $P < 0.001$) and difference between treatment means was determined using the Tukey's Studentized Range (HSD) Test. Means followed by the same letter are not significantly different.

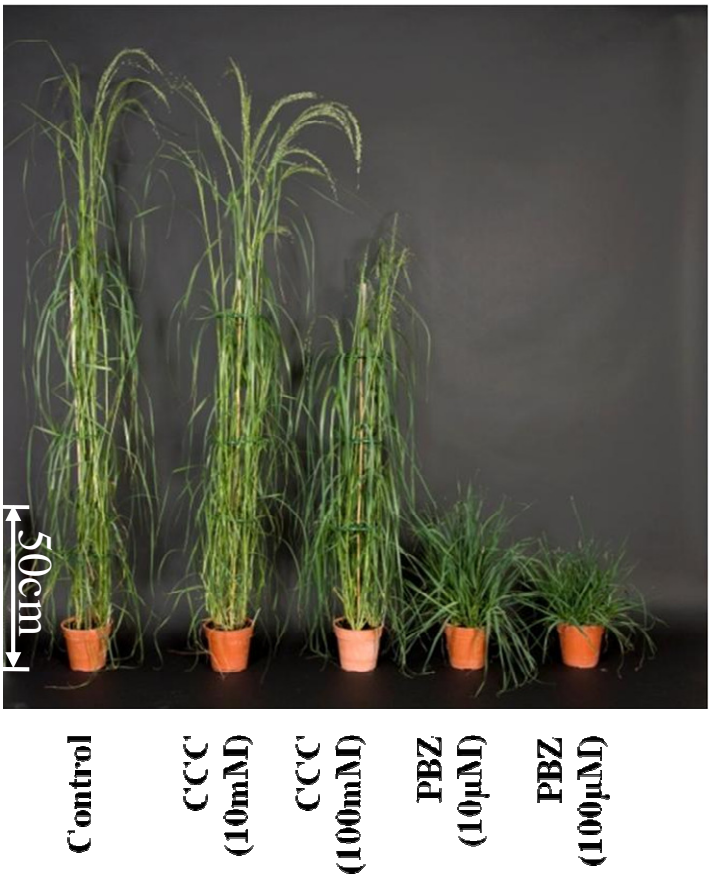

Figure 2.4 Effect of CCC (100mM) and PBZ (100µM) on plant height near plant maturity in comparison to the untreated control.

The internode diameter was, however, unaffected by either PBZ or CCC treatment except for the first internode after 10 mM CCC treatment, internodes 5 and 6 after 10 µM PBZ treatment and internode 4 after 100 µM PBZ treatment. In CCC-treated and control plants, the internode diameter steadily increased from the base up to internode 6. This indicated a poor tapering characteristic of tef plants under the selected growth conditions (Table 2.4).

Figure 2.5 Comparison of plant height and panicle growth at plant maturity as affected by PGRs (CCC: 100mM and PBZ: 10µM) application.

2.4.2.3 *Tillering, above ground biomass and yield*

PBZ-treated plants had a three-fold increase in the number of tillers per plant whereas CCC-treated plants had no significant increase in the number of tillers when compared to the untreated control (Table 2.2). PBZ treatment also significantly reduced culm and panicle dry

Table 2.3 Effects of CCC and PBZ on length of different internodes of tef var. DZ-01-196.

	Internode length (cm)							
Treatment	**I-1**	**I-2**	**I-3**	**I-4**	**I-5**	**I-6**	**I-7**	**I-8**
Control	8.6±0.9a	14.3±0.85a	18.7±0.5a	18.3±1.1a	21.9±0.9a	23.0±2.0a	27.2±1.1a	28.4±3.3a
CCC								
10mM	8.1±0.9a	12.3±0.5b	15.1±0.9b	17.3±0.8a	20.4±0.1b	22.7±0.9a	24.1±0.9b	25.0±2.7a
100mM	3.3±0.6b	8.9±0.6c	12.8±0.7c	15.8±0.5b	17.0±0.6c	18.3±0.8b	20.5±0.7c	27.6±1.3a
PBZ								
10µM	1.3±0.5c	2.0±0.5d	3.0±0.5d	4.0±0.7c	1.7±0.8d	0.8±0.1c		
100µM	0.45±0.1d	0.9±0.3e	1.0±0.3e	0.8±0.2d				
Significance	***	***	***	***	***	**	**	NS

Letters within the column denote significance as determined using the Student's *t*-test. Data shown represent mean values ±SE from 12 individual plants. Significance level was determined using ANOVA (** $P < 0.01$; *** $P<0.001$; NS = not significant) and difference between treatment means was determined using the Tukey's Studentized Range (HSD) Test. Means followed by the same letter are not significantly different.

Table 2.4 Effect of CCC and PBZ on diameter of different internodes of tef var. DZ-01-196.

Treatment	Internode diameter (mm)							
	I-1	**I-2**	**I-3**	**I-4**	**I-5**	**I-6**	**I-7**	**I-8**
Control	3.2±0.1a	3.7±0.1a	4.2±0.2a	4.34±0.2a	4.6±0.3a	4.8±0.3a	4.7±0.3a	4.0±0.3a
CCC								
10mM	2.8±0.1b	3.5±0.1a	3.9±0.1a	4.25±0.1a	4.6±0.1a	4.9±0.1a	4.9±0.1a	4.2±0.4a
100mM	3.2±0.1a	3.9±0.12	4.1±0.1a	4.22±0.1a	4.4±0.1a	4.4±0.1b	4.4±0.2a	3.8±0.2a
PBZ								
10µM	3.7±0.5a	3.9±0.4a	3.7±0.6a	3.66±0.6a	2.0±0.8b	0.8±0.6c		
100µM	3.8±0.5a	4.1±0.4a	3.7±0.6a	0.56±0.4b				
Signif.	***	NS	NS	**	*	**	NS	NS

Letters within the column denote significance as determined using the Student's *t*-test. Data shown represent mean values ±SE from 12 individual plants. Significance level was determined using ANOVA (* P <0.05; ** P < 0.01; *** P<0.001; NS = not significant) and difference between treatment means was determined using the Tukey's Studentized Range (HSD) Test. Means followed by the same letter are not significantly different.

weight in both the primary and secondary tillers when compared to CCC treated plants or to the untreated control (Table 2.6). PBZ also significantly suppressed above-ground shoot dry weight by 43.2% and 75.9% at 10 µM and 100 µM PBZ, respectively (Table 2.5; Fig. 2.6). In plants treated with 10 mM CCC secondary tiller culm and panicle dry weights were increased significantly as was total above-ground shoot dry weight when compared to untreated plants and those treated with PBZ l. In CCC-treated plants, the change in the above-ground shoot dry weight was all due either to the increase (at 10 mM) or the decrease (at 100 mM) in secondary tiller growth.

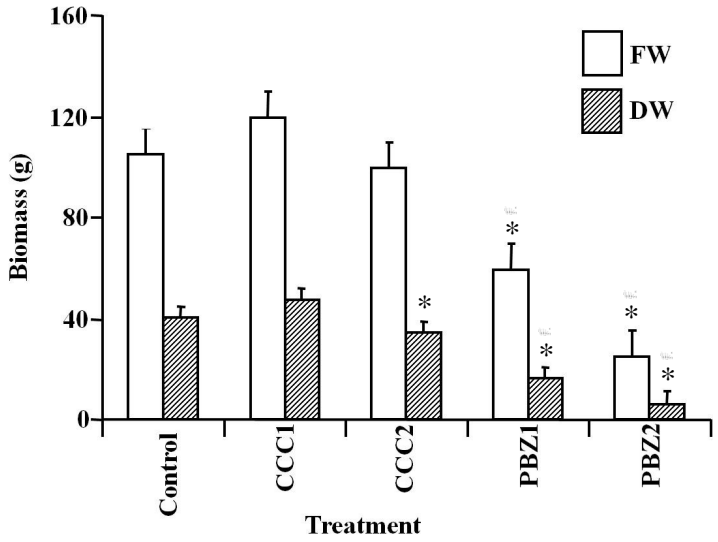

Figure 2.6 Effect of different concentrations of CCC and PBZ on biomass: fresh weight (FW) or dry weight (DW) per plant in comparison to biomass of untreated control plants. Data represent the mean ± SE of 12 individual plants. (CCC1 = 10 mM CCC, CCC2 =100 mM CCC, PBZ1 =10 mM PBZ and PBZ2 = 100 mM PBZ).

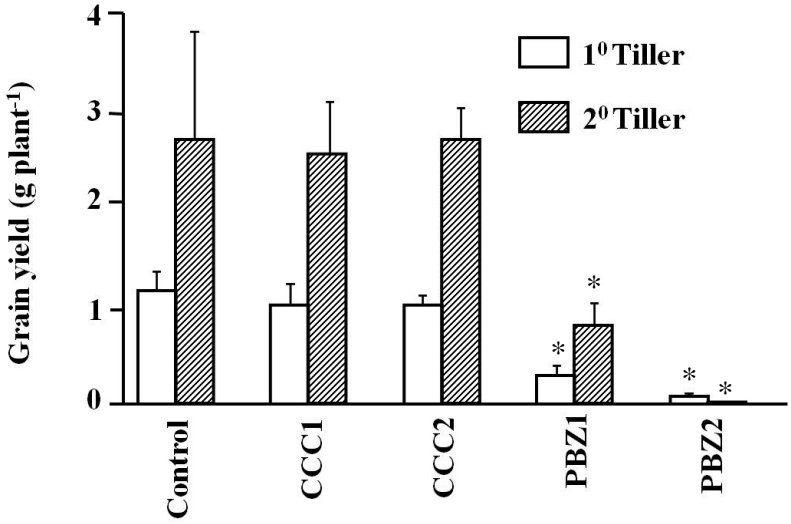

Figure 2.7 Effect of different concentrations of CCC and PBZ on primary (1^{o}) and secondary (2^{o}) tiller grain yield per plant in comparison to the untreated control. Data represent the mean ± SE of tiller grain yield of 12 individual plants. (CCC1 = 10 mM CCC, CCC2 =100 mM CCC, PBZ1= 10 mM PBZ and PBZ2 = 100 mM PBZ).

Further, in GA inhibitor-treated plants, panicle dry weight contributed less (by 8.4 - 14.5%) to above ground shoot dry weight while for panicle in the untreated control was higher (12.0%). Also, in all GA inhibitor-treated plants the tiller number per plant was negatively correlated with above-ground shoot dry weight but positively correlated with the ratio of dry mater to shoot height (Table 5). PBZ treatment (10 µM and 100 µM) significantly reduced the seed weight per plant when compared to the untreated control. Such a significant reduction was not found after CCC treatment. In CCC treated plants panicle bearing secondary tillers further contributed 63.4% to the total yield per plant (Fig. 2.7). This indicates that PBZ-induced profuse tillering, resulting mainly in non-

panicle bearing tillers, which did not contribute to grain production. Nevertheless PBZ had a stronger effect on culm than on panicle length and also clearly demonstrated that the responsiveness of *E. tef* to GA inhibition has been different between culm and panicle (Fig. 2.8).

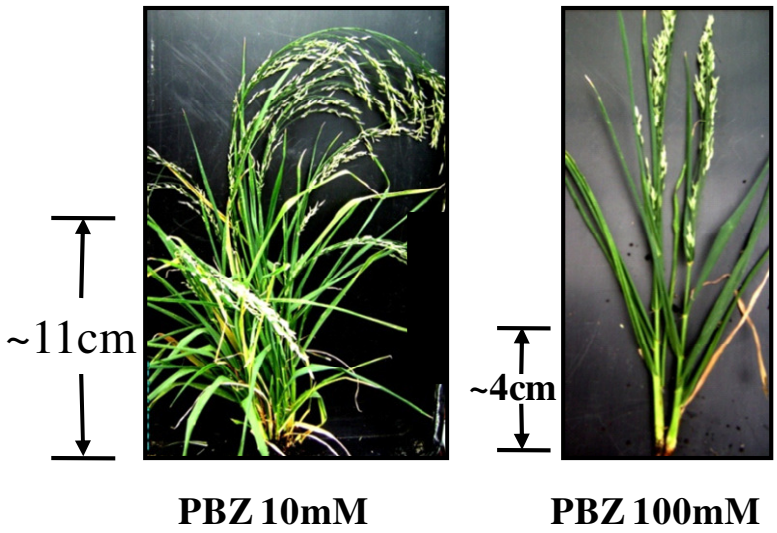

Figure 2.8 Comparison of panicle elongation with PBZ treatments in proportion to culm reduction in DZ-01-196.

Table 2.5 Effect of CCC and PBZ on dry weight of culm and panicle tillers of tef var. DZ-01-196.

	Primary tiller (g)		Secondary tiller (g)		Total shoot (g)
Treatment	**Culm**	**Panicle**	**Culm**	**Panicle**	
Control	7.11±0.3a	2.01±0.2a	25.67±1.8b	5.52±0.6b	40.30±1.9b
CCC					
10mM	6.70±0.3a	2.17±0.2a	31.10±1.3a	6.93±0.6a	46.89±1.7a
100mM	4.60±0.3b	1.87±0.1a	19.89±1.6c	6.51±0.6ab	34.53±1.9c
PBZ					
10µM	0.72±0.1c	0.73±0.2b	11.81±1.2d	2.82±0.6c	16.10±1.8d
100µM	0.41±0.1d	0.31±0.1c	5.63±0.3e	0.29±0.1d	6.41±0.4e
Significance	**	**	**	**	**

Letters within the row denote significance as determined using the Student's *t*-test. Data shown represent mean values ±SE from 12 individual plants. Significance level was determined using ANOVA (** $P < 0.01$) and difference between treatment means was determined using the Tukey's Studentized Range (HSD) Test. Means within a column followed by the same letter are not significantly different.

Table 2.6 Correlation coefficients for morphological and yield components of tef var. DZ-01-196.

	PL	PaL	TH	IN	NT	CDW	PDW	TSDW	DW/Ht	S/P
CL	0.5352	0.9281	0.9872	0.9291	-0.6415	0.8872	0.7779	0.8966	-0.8687	0.7191
PL		0.5555	0.5663	0.5579	-0.2571	0.5012	0.4383	0.5065	-0.4561	0.4557
PaL			0.9511	0.9276	-0.6028	0.8356	0.8251	0.8748	-0.8585	0.7587
TH				0.9321	-0.6145	0.8609	0.7859	0.8833	-0.8848	0.7538
IN					-0.6252	0.8486	0.7447	0.8634	-0.8532	0.6522
NT						-0.4258	-0.4248	-0.4307	0.8029	-0.4401
CDW							0.7904	0.9803	-0.7199	0.6682
PDW								0.8809	-0.6543	0.8923
TSDW									-0.7372	0.7511
DW/Ht										0.9869

CL = Culm length; PL = Peduncle length; PaL = Panicle length; TH= Total height; IN= Internode number; NT = No. of tillers; SFW= shoot fresh weight; CDW = Culm dry weight; PDW = Panicle dry weight; SDW = Shoot dry weight; DW/Ht = shoot dry weight per height ratio; S/P = seed weight per plant. Significance level for the Pearson Correlation Coefficients was determined using the SAS statistical package and all values are significant at $P<0.001$.

2.5 Discussion

Results have shown a GA-dependent control of plant height in tef. Application of GA_3 (10^{-2}M) increased plant height by 41% in plants of the short genotype and by 15% in the tall genotype under greenhouse conditions. Applying CCC at 10^{-2}M reduced plant height by about 43% in the tall genotype DZ-01-196 when compared to a 21% reduction in the short genotype Gea Lammie during the six weeks of plant growth. Application of the GA inhibitor CCC reduced the plant height in plants of DZ-01-196 (tall phenotype). This effect was more dramatic than the effect on plants height in Gea Lammie. Since GA increased height in Gea Lammie and CCC reduced height in both, the short phenotype in Gea Lammie is not considered to be either due to interference in the GA-biosynthesis or GA- response but could be associated to other factors not relevant to GA metabolic genes. Studies in wheat has shown that tall and intermediate varieties showed a greater response to CCC than the short or semi-dwarf varieties containing mutant GA genes like *Rht1/Rht1* (Börner & Meinel, 2006; Abbo *et al*., 2004).

The content of the bioactive GA_1 and GA_3 and all the precursors were higher in the tall genotype, DZ-01-196, than in Gea Lammie showing in most cases about a two fold increase. Thus the bioactive GA concentration was a good reflection of the difference in plant height between the two genotypes. This also explains the differential responses to exogenous GA_3 application where DZ-01-196 was less responsive to exogenous GA treatment compared with Gea Lammie because of higher bioactive GA amount in the plant tissue.

Further study using only the tall variety (Experiment II) has demonstrated that the GA inhibitors CCC and PBZ reduce stem growth in tef but with a much stronger effect of PBZ when compared to CCC. Culm length was the most responsive plant part to inhibitor action and CCC reduced culm length by one quarter but without reducing panicle growth and grain yield.

Based on our finding that CCC treatment reduces plant height in *E. tef*, the PGR effect on DZ-01-196 was investigated in greater detail because this variety has been widely grown for its high yield and grain quality. It is also used as a parental line in *E. tef* breeding program but suffered from lodging losses (Yu *et al.* 2007; Assefa *et al.* 2010). Therefore, effect of GA inhibitors CCC and PBZ on DZ-01-196 was specifically studied further. Both GA inhibitors CCC and PBZ significantly reduced stem growth in DZ-01-196 but with a much stronger effect of PBZ at a much lower concentration when compared with CCC because of its stronger inhibitory effect on GA biosynthesis than CCC (Lurie *et al*., 1997)). Culm length was most responsive and CCC reduced culm length almost by a quarter without reducing panicle growth and grain yield A further reduction of culm height might be achieved by increasing CCC concentration, but such increased concentration should not affect panicle growth. Yield increase associated with CCC application, reported for other crops (Berry *et al*., 2004), was not found for *E. tef*. This could be because of absence of increased head-bearing tillers following CCC application.

PBZ, greatly reduced culm elongation particularly affecting elongation of the uppermost four internodes which in most cases was completely inhibited. Also, a lower PBZ

concentration might be applied so that a less drastic effect is achieved in reducing the plant height and with minimal effect on panicle growth and yield.

Since GA inhibitor action greatly affected culm length, this indirectly imply that a higher demand for endogenous GA may exist by the elongating stem than by the panicle. This may be due to a higher meristematic activity and cell elongation in the intercalary meristem of the elongating stem rendering it more sensitive to changes in the GA amount. Shortening was more pronounced in the lowermost internodes with up to a 2.6-fold reduction when compared to the control. Shortening of basal internodes, if associated with stem wall thickening or increase in dry weight per unit of basal internodes, will further minimize the lodging risk. For example, the basal internode length and plant height in wheat are the two most important culm traits closely associated with lodging (Kelbert *et al*. 2004).

It was also observed that soil-applied CCC shortened all internodes, foliar application shortened the uppermost two internodes (Gebre, *et al*., 2011). A similar effect for foliar-applied CCC has been previously also reported for barley (Sanvicente *et al*., 1999) and hybrid rye (Froment and McDonald, 1997). The results also indicate a possible differential response to mode of application which could be related to the translocation of CCC to the site of the biochemical targets being more localized in leaf application. *E. tef* may benefit more from foliar than soil application. Irregularity of responses has been noted in other crops presumably for differences in the decomposition of the chemical in the soil, depending on prevailing temperature and humidity conditions (Radmacher, 2000; Pintus, 1973).

In our study CCC treatment even increased panicle length, but this increase was not related to any change in seed weight per plant. Whereas in a previous study with wheat improved grain set thus increased harvest index was obtained following shortening of stem (Rajala, 2003). Absence of a CCC effect on *E. tef* panicles possibly indicates a low demand for bioactive endogenous GA for panicle growth and therefore inhibition of GA biosynthesis does not greatly affect yield while reducing plant height. Agronomically, this would be advantageous allowing high productivity in tef plants while minimizing lodging.

In this study, we also investigated the effect of the GA-biosynthesis inhibitors on stem diameter. In PBZ-treated plants tapering stem morphology with a steadily increasing stem diameter was found. In contrast CCC-treated and control plants only had an increase in stem diameter but in the upper internodes only. Such steady acropetal increase in stem diameter might exacerbate lodging susceptibility and this problem might even become more serious with increasing N-fertilization allowing minimum wind speed or rain to cause lodging. This also indicates that *E. tef* plants have a weak transition from shoot to root with a smaller plant-base diameter causing poor tapering. Absence of an effect on stem-base diameter by CCC is not unique to *E. tef* as it has also been reported in wheat and barley (Gendy & Hofner 1989; Berry *et al*. 2000).

PBZ-treated plants had a higher tiller number compared to CCC-treated plants. A continuous application of a high PBZ dose strongly promoted tillering but inhibited tiller elongation. This limits leaf expansion and photo-assimilation thereby reducing carbohydrate reserves required for rapid growth (Stavang *et al*., 2009). Promotion of

tillering by GA deficiency and inhibition of stem elongation has been recently also reported for other cereals (Rajala, 2003; Lo *et al*., 2008). However, in contrast to wheat, barley and oats, where increased tillering was found after foliar and seed treatment with CCC (Naylor *et al.,* 1989; Craufurd and Cartwright, 1989; Peltonen and Peltonen-Sainio, 1997; Peltonen-Sainio *et al.,* 2003), no significant increase in tillers was found in tef when treated with CCC. It has been observed, in general, that gibberellins tend to cause less development of auxiliary buds while promoting the elongation of already growing (initiated) stem as well as tillers (Peltonen and Peltonen-Sainio, 2001). On the other hand the application of GA-inhibitors promote tiller initiation and stunting of the central stem. Tillering is generally considered an adaptation to environmental changes. Under a long-day condition, as applied in this study, CCC treatment has been found to produce in other cereals more tillers per main shoot at maturity (Rajala, 2003). Further, in wheat a high number of tillers reduces plant productivity in terms of grain production and lodging resistance and it has been further suggested that a low tiller number per m^2 is required for lodging resistance (Tripathi *et al*., 2003). In tef breeding, however, the main focus has been on obtaining high tillering with tall plants for improved grain yield. It would be therefore important for developing a lodging resistance tef ideotype where plant height is differentially controlled (decoupling of plant height and yield) while maintaining optimum number of panicle bearing tillers that is yet to be determined from the lodging stand point.

In *E. tef* breeding, taller plants with higher number of tillers have been the main focus for grain yield improvement. In this study, it has been demonstrated that CCC can reduce plant height without affecting grain yield. It is therefore important to develop a lodging-

resistant *E. tef* ideotype differentially controlling plant height and yield. Generally, this study has demonstrated that GA biosynthesis is a prime target for plant height regulation. Since CCC reduces plant height without affecting grain yield, this compound might be suitable for lodging prevention providing the advantage of high productivity. Treatment with CCC did not increase stem diameter, the diameter to height ratio however increased and CCC treatment would therefore also improve plant standability. Although PBZ is probably useful at lower concentrations, its high cost and persistence in the soil would restrict its wider application. However, future fine-tuning might be required to optimize CCC use for commercial application in lodging prevention without compromising seed yield. However, extensive use of chemicals such as CCC may not be sustainable for environmental concerns. In which case long term solutions through genetic modification of GA metabolism and targeting genes, such as the rice *sd-1* and the wheat *Rht* orthologs in *E. tef*, could be a strategy for plant height control in *E. tef*.