

CHAPTER 5

EVALAUTION AND ANALYSIS OF MUTANT TEF (*Eragrostis tef*) LINES FOR DWARFISM FOR LODGING RESISTANCE

5.1 Abstract

To evaluate *E. tef* plants generated via mutagenesis to induce dwarfism, selected mutant lines were evaluated for traits including culm height and diameter, internode number and length, panicle length, shoot biomass, tillering and grain yield under GA sprayed and non-sprayed conditions. Semi-dwarfed phenotypes could be developed in *E. tef* through mutagenesis approach and culm height was significantly reduced (23.1 cm - 41.7 cm) in three mutant lines. These mutants were semi-dwarfed with short culm and peduncle length. Regardless of height, grain yield was considerably reduced in all the mutants showing severe defects in fertility except mutant GA-10 which gave a reasonable yield. Line GA-10 also had a significantly higher diameter at 2nd - 5th and at 7th internodes contributing to the stiffness of the stem and also had the highest panicle dry weight among mutant lines tested. Internode diameter showed consistent increase acropetally with weak tapering. All semi-dwarfed mutants did not respond to GA treatment. Plants of G-10 possibly harbours a mutation in GA signalling. Since biomass production in mutant line GA-10 was not reduced this line might be used for crossings with other parental lines to restore yield without losing its other useful traits.

5.2 Introduction

Several studies have shown morphological traits that are related to the lodging in *E. tef* to be related to plant height, stem diameter of lower internodes, panicle length, biomass and seed weight (Chanyalew, 2010; Hundera *et al.* 1999; Ketema, 1983; Mengesha *et al.* 1965). Considerable efforts have been made over the last 50 years to incorporate by conventional breeding desirable agronomic traits into *E. tef*. However, no lodging resistance traits, such as reduced height and stiff straw, have been so far reported using conventional breeding (Assefa *et al.*, 2010).

Mutation breeding has been carried out in cereals to induce semi-dwarfness (Narahari, 1985) and also to solve the lodging problem (Maluszynski and Szarejko, 2003). In general, inducing mutation in target genes using various mutagens provides rapid generation and enhancement of genetic variability (Nichterlein, 2000). Short stature mutants without changing the background character of important traits have been beneficial not only to improve lodging resistance but also to increase productivity. This has been possible because of a more efficient partitioning of the dry matter resulting in high harvest index and increased grain yields in dwarf mutants in cereals due to pleiotropic effects (Hanson *et al.*, 1982; Hu, 1973; Nichterlein, 2000). In *indica* type rice, a spontaneous mutation resulting in semi-dwarfs led to the development of the high-yielding variety 'IR8'. This variety, together with its descendants, was responsible for the Green Revolution in Asia. More than 60 dwarf or semi-dwarf mutant lines have been so far reported for rice. Some of these are allelic to *sd1*, but no mutation, other than in the *sd1* locus, resulted in improvement of agronomic performance. In barley several lines with induced mutation has

resulted in superior breeding material (Maluszynski and Szarejko, 2003) and in wheat, semi-dwarf phenotypes have been obtained through mutation in the *Rht* loci.

Recently a TILLING (Targeting Induced Local Lesion IN Genomes) approach has been carried out for *E. tef* to generate variability in plant height and to develop semi-dwarf phenotype for lodging resistance in *E. tef* (Esfeld and Tadele, 2010). In this part of the overall study, the first objective was to select mutant lines from the TILLING M3 population to evaluate them for morphological (plant stature) and yield traits to possibly identify any desirable mutation for lodging resistance development in *E. tef*. The second objective was to characterize potential candidate mutant lines for changes in the DNA sequence of particular target genes (*GA20ox* homologues and *Rht*).

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5.3 Materials and Methods

5.3.1 Plant material

Seed material of mutagenized *tef* (*Eragrostis tef*) lines (M3 progenies) of variety DZ-Cr-37 (fairly tall) was obtained from Dr. Zerihun Tadele, University of Bern, Switzerland.

5.3.2 Plant growth and GA treatment

Plant growth experiments were carried out in an environmentally controlled greenhouse at Rothamsted Research, UK, from May to August 2010. Up to 36 seeds per each mutated line (9

per pot) were germinated and thinned down to 3 per pot and grown on a compost mix consisting of peat (75%), sterilized loam (12%), vermiculite (3%) and grit (10%). The mix was supplemented with a slow releasing fertilizer containing 15-11-13 NPK plus micronutrients. Selected seedlings were maintained in the same pot [15 cm diameter (top) x 12.5 cm (height) and 10 cm (bottom)] maintaining 3 seedlings per pot. Since these mutants were from M3 seeds (successively selfed plants), uniformity was kept among the segregating plants by removing seedlings with phenotypes that resembled the wild-type. When the mutant phenotype was very close to the wild-type, selection was not possible and seedlings were selected at random. Six plants per treatment were hand-sprayed with 100 μM GA3 every week on the surface of the leaves. Seedlings were grown for 14 wks until plant maturity in an environmentally controlled greenhouse using a 16 h photo-period provided by natural light supplemented with light from sodium lamps to maintain a minimum PAR of 350 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

5.3.2.1 Growth measurements

Culm length was measured from main culms from the ground to the base of the spike. Culm diameter was measured at harvest time from the main culm at the basal internode with a digital calliper. Grain yield and shoot biomass were measured for each plant in each replication. Panicle length of main tillers was measured from the node, where the lower base emerges above the peduncle. Tiller height was determined by summing the culm height as well as peduncle and panicle lengths. Internode and tiller number were determined by counting the number of internodes or tillers per plant. Internode diameter was measured about 3 mm above the each node using a standard digital calliper. Dry weight was determined from above-ground plant material

by drying fresh material at 80°C for 2 days in an oven. Grain yield was determined by measuring the weight of seeds from all tillers.

5.3.2.2 Data analysis

Growth and yield data were analyzed using the SAS statistical package (SAS Institute Inc., Cary, NC, USA) for Analysis of Variance (ANOVA) and Pearson Correlation Coefficients. 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.

5.4 Results

5.4.1 Culm height, internode length and diameter

Three lines (3-7-4852-1, 3-7-315-12 and GA-10) had significantly shorter ($p < 0.05$) and two lines (3-7-4682 and 3-7-5160) had significantly ($p < 0.05$) taller culm length than the wild-type plants (Fig. 5.1). Most dwarfed lines (3-7-4852-1, 3-7-315-12 and GA-10) were not significantly different ($p > 0.05$) from each other in culm length despite a 18.5cm culm length difference between them. Culm height difference between plants of the tallest and shortest mutagenized lines was about 70cm (Fig. 5.1). Decrease in culm length in plants of the three shorter lines derived from either a decrease in internode number or length or a decrease in peduncle length (Figs. 5.1 and Table 5.3). Lines 3-7-5131-2 and 3-7-4852-1 gave significantly ($p < 0.05$) higher number of internodes (8.3) when compared to wild-type plants (6.9; Table 5.1).

Application of GA did not significantly increase ($p > 0.05$) culm or internode length in plants of the three dwarfed lines (3-7-4852-1, 3-7-315-12 and GA-10) when compared to the wild-type control. GA treatment further did not significantly ($p > 0.05$) affect peduncle and panicle length including the wild-type control. Dwarfed plants of mutant lines did not significantly ($p > 0.05$) change culm, panicle or peduncle length when treated with GA (Figs. 5.2 and 5.3). Also, mutant plants of line GA-10 had a significant ($p < 0.05$) increase in internode diameter when compared to wild-type plants when either treated or not treated with GA₃ (Table 5.4). Further, internode diameter had a slight, but steady, increase upward in plants of all mutant lines (Table 5.4).

Table 5.1 Peduncle length, internode number, number of tillers, culm and panicle dry weight of plants of different mutant *E. tef* lines and wild-type control (variety DZ-Cr-37).

Line	Peduncle length (cm)	Internode number	Tiller number	Culm + Leaf DWT (g)	Panicle DWT (g)
DZ-Cr-37					
(wild-type)	29.92a	6.8bc	13.08dc	25.8b	9.60a
3-7-4682-2	24.58ab	7.5ab	13.08dc	26.8b	9.12a
3-7-5160-1	25.72ab	6.9bc	16.23bc	32.5a	3.72c
3-7-5131-2	27.65ab	8.3a	10.23d	25.1b	5.06b
3-7-4852-1	13.08d	8.3a	20.75a	15.1c	0.31d
3-7-315-12	18.04cd	7.4abc	18.73ab	10.3d	0.40d
GA-10	23.67cb	6.4c	14.42c	25.5b	9.49a
Mean	23.24	7.37	15.22	23.1	10.22
P	***	**	***	***	***

DWT= dry weight; Letters within a column denote significance as determined by the Tukey's Studentized Range (HSD) test. Data shown represent mean values \pm SE of 12 individual plants. Significance level was determined using ANOVA (*** $P < 0.001$; ** $P < 0.01$). Means followed by the same letter are not significantly different.

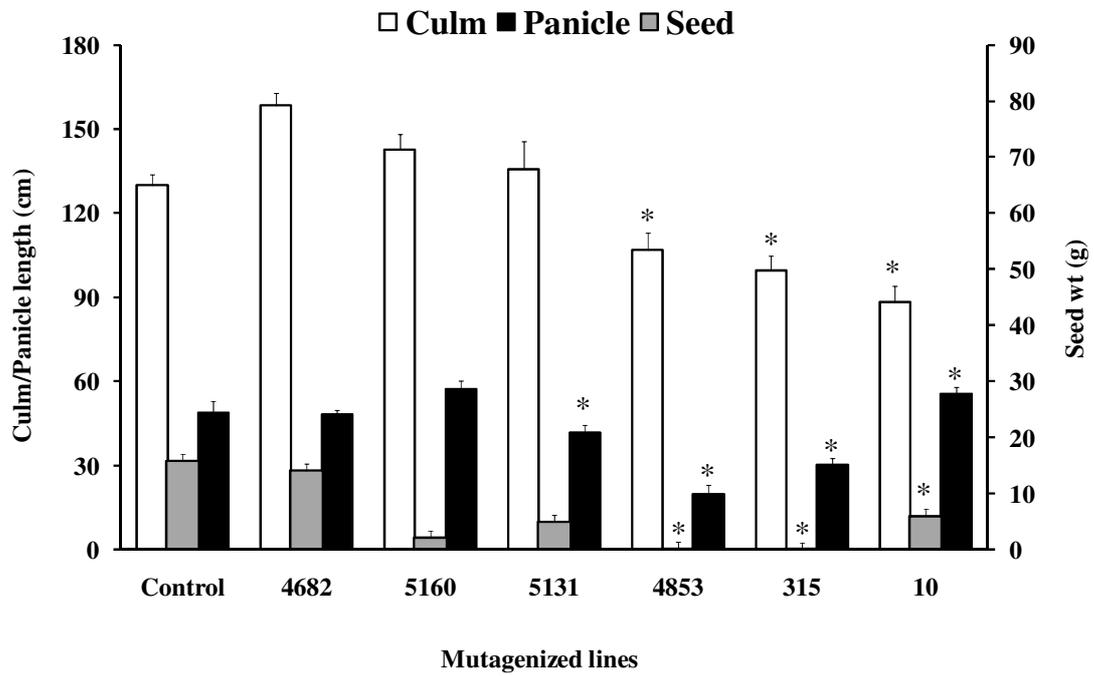


Figure 5.1 Culm and panicle length and seed weight in plants of different mutant lines and the control (DZ-Cr-37). Data represent the mean \pm SE of 12 individual plants.

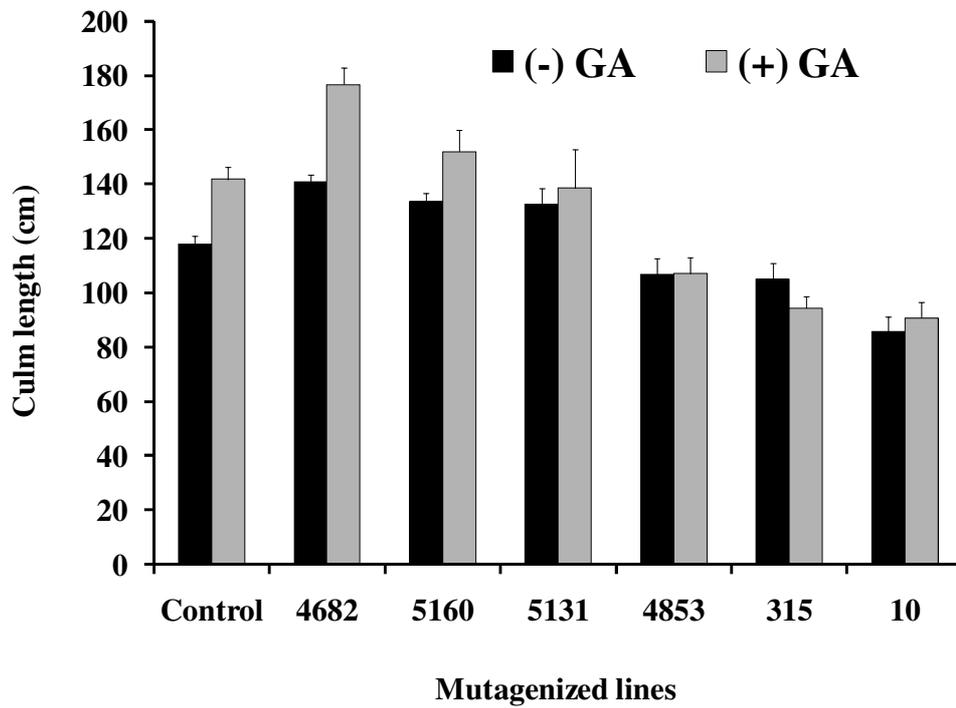


Figure 5.2 Culm length of plants of different mutagenized lines and wild-type plants (DZ-Cr-37) with (+) GA and without (-) GA treatment. Data represent the mean \pm SE of 6 individual plants.

5.4.2 Panicle length, tillering, biomass and yield

Among the plants of the three dwarf mutant lines with the highest reduction in culm length, plants of lines 3-7-4852-1 and 3-7-315-12 had significantly ($p < 0.05$) lower panicle length when compared to wild-type control plants. However, plants of the most dwarfed mutant line GA-10 had an identical panicle length when compared to the wild-type control (Fig. 5.1) regardless of additional GA treatment (Fig. 5.3). Plants of the two mutant lines 3-7-4853 and 3-7-315 had further the shortest panicle length (Fig. 5.1) and plants of these lines had also severe defects in fertility and produced only little grain.

Tillering in plants of the two mutant lines (3-7-4852-1 and 3-7-315-12) was further significantly ($p < 0.05$) higher than in plants of the shortest mutant line (GA-10) and the wild-type control (Table 5.1). Tillering was also not reduced by GA treatment (Table 5.3) and tiller number was also not related to culm length (data not shown).

Plants of the two mutant lines with reduced height (3-7-4852-1 and 3-7-315-12) had significantly ($p < 0.05$) lower panicle dry weight and reduced yield when compared to the control or all other mutant lines (Table 5.3). Plants of mutant line GA-10 produced about half the yield (2.5 g) of the wild-type control (4.8 g). In all plants of dwarf mutant lines yield was not increased by GA treatment (Table 5.3).

Table 5.2. Response of mutant lines in terms of internode length to exogenously applied GA₃

GA	INT1		INT2		INT3		INT4		INT5		INT6		INT7		INT8		INT9	
	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Wild type	6.7	12.1	14.6	16.5	16.4	22.6	18.3	20.4	20.6	24.1	20.2	27.8	21.2	27.5	-	-	-	-
3-7-4682-2	10.2	11.5	18.7	16.3	17.8	18.0	18.8	24.8	22.3	24.1	24.1	23.3	28.6	27.3	-	23.7	-	23.0
3-7-5160-1	9.1	13.0	15.8	18.9	19.6	22.1	18.1	21.8	24.1	27.9	26.3	27.9	29.4	26.1	-	-	-	-
3-7-5131-2	7.7	7.1	15.4	9.46	15.8	15.8	16.3	17.0	19.9	17.3	24.0	20.9	25.6	15.6	25.0	11.4	-	16.5
3-7-315-12	5.3	6.5	10.5	8.8	13.0	10.6	15.5	14.9	18.5	16.1	17.8	16.2	16.6	13.1	17.1	15.3	-	-
3-7-4852-1	3.6	8.1	6.8	10.8	8.8	12.1	9.5	14.0	10.1	14.0	13.1	19.7	15.6	16.8	17.1	12.5	-	-
GA-10	5.1	4.1	9.58	9.4	12.4	12.5	13.8	13.7	16.1	18.3	18.1	19.7	20.6	23.7	-	-	-	-
GA Mean	7.0b	8.8a	13.3a	13.2a	15.4a	16.4a	16.5b	18.6a	19.5a	20.8a	21.2a	22.2	23.1a	21.8a	19.7a	17.9b	15.8a	19.7a
GA	*		NS		NS		**		NS		NS		NS		***		NS	
LinexGA	NS		*		*		NS		NS		**		**		NS		NS	

Significance level was determined using SAS GLM for ANOVA (** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$); L!= Line; Letters for mean denote significance as determined using the Tukey's Studentized Range (HSD) Test. Data shown represent mean values \pm SE of 6 individual plants. Means followed by the same letter are not significantly different. NS = Non-significant; (-) = control or unsprayed; (+) = Sprayed with GA₃ (100uM).

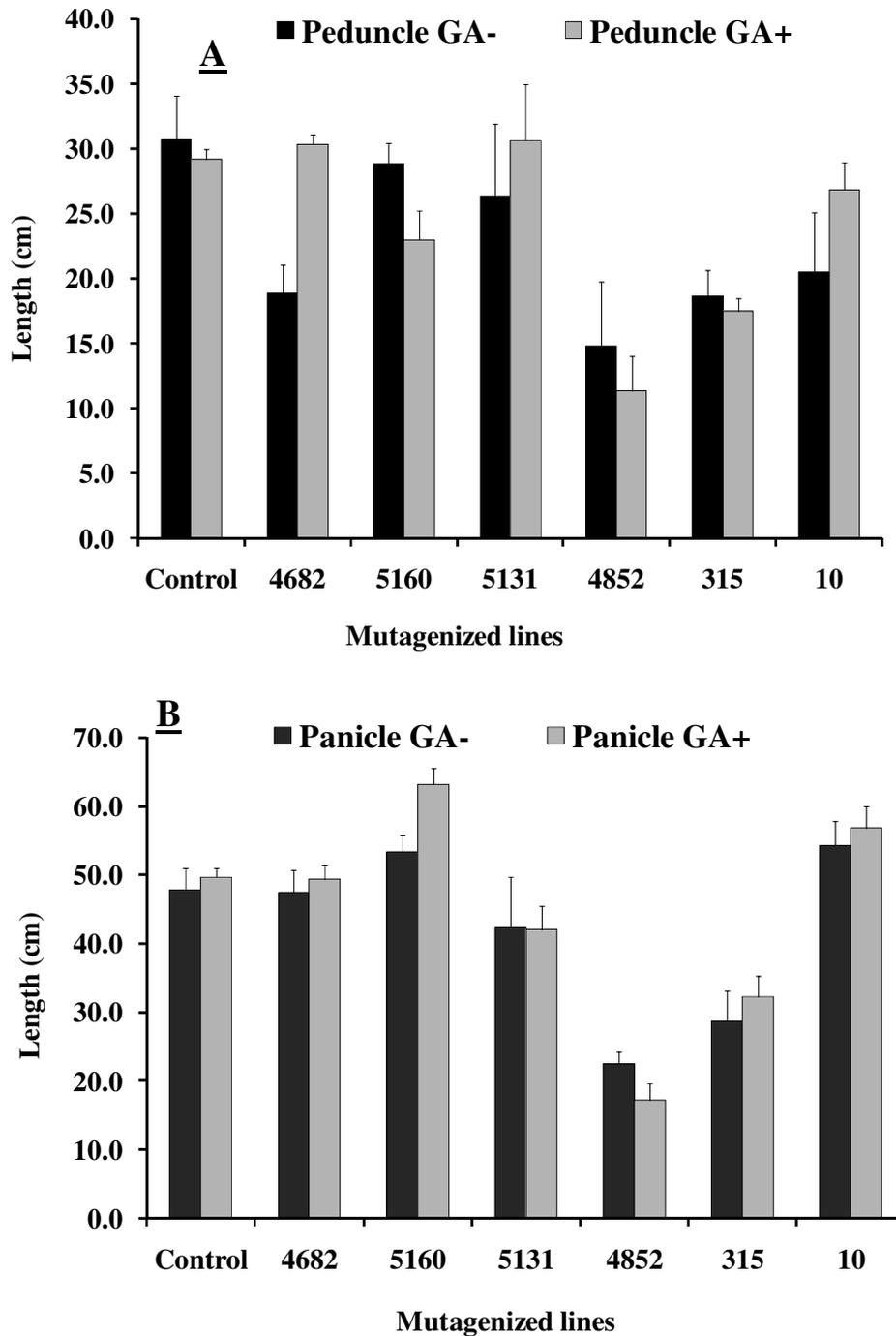


Figure 5.3 Effect of GA application on peduncle (A) and panicle (B) length of mutant lines and the control (DZ-Cr-37). Data represent the mean \pm SE of 6 individual plants.

Table 5.3 Peduncle length, internode number, number of tillers, culm and panicle dry weight of plants of different mutagenized *E. tef* lines and wild-type control plants (cv. DZ-Cr-37).

Line	Internode number		Tiller number		Culm DWT (g)		Panicle DWT (g)		Seed WT (g)	
	GA-	+	-	+	-	+	-	+	-	+
Wild-type	7.0	6.5	13.5	12.7	26.1	25.4	10.2	8.9	4.9	4.6
3-7-4682-2	7.0	8.0	12.7	13.5	26.4	27.2	9.1	9.1	4.5	4.9
3-7-5160-1	6.7	7.2	15.8	16.4	33.8	31.2	3.4	4.1	0.4	1.1
3-7-5131-2	7.3	9.2	8.7	11.8	21.6	29.3	4.3	5.9	1.3	2.4
3-7-4852-1	9.2	7.5	22.7	18.8	14.2	15.9	0.4	0.3	0.07	0.08
3-7-315-12	7.3	7.4	21.7	14.6	11.4	9.1	0.3	0.5	0.00	0.01
GA-10	6.5	6.3	13.7	15.2	24.7	26.2	8.8	10.2	2.2	2.8
Mean	7.3a	7.3a	15.2a	14.6a	22.9a	23.3a	5.60a	6.10a	2.25a	2.70a
GA	NS		NS		NS		NS		NS	
Line x GA	***		NS		***		***		***	

DWT= dry weight; Significance level was determined using SAS GLM or ANOVA (*** $P < 0.001$). Letters for mean denote significance as determined using the Tukey's Studentized Range (HSD) test. Data shown represent mean values \pm SE of 6 individual plants. Means followed by the same letter are not significantly different. NS = Non-significant; GA (-) = control or unsprayed; GA (+) = sprayed with gibberellic acid (GA_3 at 100 μ M).

Table 5.4 Internode diameter of different mutant lines and wild-type control (cv. DZ-Cr-37) plants in response to GA₃ treatment.

Line	INTØ1*		INTØ2		INTØ3		INTØ4		INTØ5		INTØ6		INTØ7		INTØ8		INTØ9		
	GA-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	
Wild type	1.84	1.55	2.30	1.92	2.50	2.44	2.74	2.51	2.89	2.66	3.06	3.02	3.29	3.16	-	-	-	-	
3-7-4682-2	1.97	1.61	2.67	1.43	2.95	2.24	3.04	2.73	3.48	2.74	3.42	2.80	3.65	2.83	-	2.52	-	1.98	
3-7-5160-1	2.08	1.59	2.61	1.80	2.80	2.46	3.15	2.68	3.11	2.78	3.44	3.06	3.86	3.13	-	-	-	-	
3-7-5131-2	1.93	1.52	2.25	1.86	2.89	2.34	3.23	2.57	3.28	2.69	3.84	2.88	3.92	2.84	3.63	3.02	-	1.92	
3-7-4852-1	1.41	1.02	1.44	1.13	1.42	1.26	1.61	1.51	1.61	1.84	1.71	1.83	1.61	1.55	1.55	1.30	-	-	
3-7-315-12	1.50	0.77	1.87	1.09	2.03	1.40	2.03	1.41	2.33	2.02	2.32	1.83	2.54	1.54	2.26	1.28	-	-	
GA-10	1.90	1.85	2.74	2.45	2.86	2.80	3.24	2.95	3.55	3.11	3.24	3.34	3.71	2.90	-	-	-	-	
Mean	1.82a	1.43b	2.28a	1.69b	2.51a	2.14b	2.74a	2.34b	2.92a	2.54b	3.01a	2.69b	3.19a	2.58b	2.48a	2.11a	1.44a	1.74a	
GA	***		***		***		***		***		***		***		***		**		NS
Line x GA	NS		*		NS		NS												

*IntØ1= internode No. 1 diameter from the base of the stem; Significance level was determined using SAS GLM for ANOVA (** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$). Letters for mean denote significance as determined using the Tukey's Studentized Range (HSD) test. Data shown represent mean values \pm SE of 6 individual plants. Means followed by the same letter are not significantly different. NS = Non-significant; GA (-) = control or unsprayed; GA (+) = sprayed with gibberellic acid (GA₃ at 100 μ M).

5.5 Discussion

This study has shown that semi-dwarf phenotypes could be developed in *E. tef* through mutagenesis. However, regardless of plant height grain yield was considerably reduced in all the mutant lines, possibly because of other undesirable effects induced in the plants. Some of the mutants showing considerable reduction in height were also found near infertile and produced only a very little amount of grain except for plants of the most dwarfed mutant line GA-10, which gave about half of the untreated wild type control..

Internodal diameter was not increased due to induced semi-dwarf phenotype with the exception of semi-dwarf line GA-10 which had consistently higher internodal diameter from 2nd to the 5th internode in the GA untreated plants compared to GA untreated wild type control. Such increase in internode diameter contributes to the stiffness of straw (stem) by increasing the ratio for stem basal-diameter to height which would be of benefit for obtaining lodging resistance. On the other hand, the weak tapering of control plants could not be improved in any of the dwarf mutant plants since acropetally steadily increasing internodal diameter was found in all of the mutant plants. Based also on our previous results from GA inhibition studies (Chapter 2) that plant height reduction did not increase stem basal diameter, results of this study also confirmed absence of meaningful relationship between plant height and stem diameter. However, this is under the assumption that the induced semi-dwarf phenotype in the mutant plants in this study also involves mutation in the height controlling genes. This, therefore, is subject to further verification. Therefore, it is possible to speculate that factors, other than GA genes mediated controlling of culm height might also be involved in controlling internode diameter. This speculation is in line with the recent report by Ookawa *et al.* (2010) about enhanced stem strength obtained in japonica rice plants carrying

the *STRONG CULM2* (*SCM2*) gene due to its effect on increasing culm diameter. Therefore, mutagenesis and TILLING application can also be directed to induce change in such genes involved in culm diameter control for lodging resistance improvement. It, however, is not yet clear from the present study if mutant line GA-10 may harbor induced changes in such genes involved in stem diameter control.

None of the semi-dwarf mutant including GA-10 plants showed a response to GA treatment and did not recover height. This means the induced changes are not in the GA biosynthesis since such mutants can easily recover height by exogenous GA application. The alternative is change in GA signalling in which case studies have already shown that GA signalling mutants are insensitive to GA treatment (Milach *et al.*, 2002; Hedden *et al.*, 1998). It might, therefore, be possible that mutant plants not responding to exogenously applied GA harbour a mutation in GA signalling components such as the *Rht* in GA metabolism.

Plants of semi-dwarf lines also had normal tillering but did not have identical biomass amount as the wild-type control except mutant line GA-10. Hence, most tillers were rather weak with slender stem having little biomass to support further growth and sustain grain filling as evident from the very low yield. However, biomass in the semi-dwarf mutant GA-10 was identical to the biomass in the control plants which could be of significant advantage if this line is used for crossings with other parental lines to restore yield without losing its other useful traits such as the semi-dwarf and higher internodal diameter. Plants of GA-10 were non-responsive to GA application implying that it may harbor a mutation possibly in GA-response genes. It also have shown a higher GA content (data not shown: personal communication Dr. Tadelle) which also suggests an impaired GA signalling component such as changes in the *Rht* gene. GA signalling mutants have been shown to have elevated levels

of bioactive GA presumably because of a feedback regulation as a result of reduction in GA-response (Alvey and Harberd, 2005). However, it is necessary to verify the assumptions in future experiments to determine the agronomic importance of mutant line GA-10. Crossing GA-10 with known tall varieties would also be necessary to determine the line's potential as a source of a semi-dwarf trait. Evaluation can be further supplemented with characterization of the DNA sequences for target GA-signalling genes such as *Rht*.

Overall, this study has shown that a TILLING approach can be applied to obtain semi-dwarf *E. tef* plants. Application so far has resulted in different semi-dwarf lines and one of these lines, GA-10, has potential for further characterization including the cause for dwarfism.



CHAPTER SIX

GENERAL DISCUSSION AND FURTHER PERSPECTIVE

The study aimed to be investigate the role of GA in plant height control in *E. tef* and associated changes *in* morpho-physiological parameters of the plant including yield . Results showed the significance of GA genes as prime targets for plant height reduction in *E. tef* to ultimately improve lodging resistance by reduction of culm length.

A first new aspect of this study was the high responsiveness of *E. tef* in terms of plant height reduction following GA inhibition by anti-gibberellins chemical treatment (CCC and PBZ). A further new finding was that growth of the *E. tef* panicle, which constitutes one third of the total plant height, was not very sensitive to GA inhibition. At higher concentration of a potent inhibitor, such as PBZ, stem elongation was severely reduced. Generally, the control of GA inhibitors on stem height and panicle elongation provided strong evidence that targeting the GA biosynthesis pathway is a realistic strategy for the control of plant height in *E. tef*. Further, evidence was provided that decoupling plant height and yield could be achieved in *E. tef*. This would allow developing dwarf lodging-resistant plants in high yielding cultivars.

Although PBZ had a much stronger GA inhibition on internode elongation in *E. tef* than CCC and also acting at a much lower concentration than CCC, PBZ cost and persistence in the soil would restrict its wider application. In addition, panicle bearing tillers was not increased by PBZ or CCC treatment although PBZ increased tillering many-fold. Unfortunately, CCC treatment did not increase stem diameter, but the diameter to height ratio was increased improving plant standing. Therefore, CCC appears to be a suitable inhibitor and a candidate for further plant height control for reducing the lodging problem under field conditions. Fine-tuning of CCC application and observing response of plants (with reduced height) under field conditions are, however, required to prepare a practical guideline for wider application of the PGR in *E. tef*. This will help to identify a good balance between vegetative (stem and tiller)

and reproductive (panicle and seed setting) growths for effectively reducing height (therefore lodging) without compromising seed yield. The weak tapering in *E. tef* observed in this study together with absence of any promising effect on stem diameter is still a concern suggesting a weak transition between the shoot base, 1st lowermost internode and the root collar, in *E. tef* plants. Emphasis should therefore also be given to understand the mechanism, other than those regulated by GA genes, that might involve improving *E. tef* stem-base.

A further new aspect of this study was the optimization of *E. tef* regeneration to produce putative transformed plants from immature somatic embryos via *Agrobacterium*-mediated transformation for the induction of dwarfism over-expressing GA inactivating gene (*GA2ox*) from *Phaseolus coccineus*. In this study, 8 putative transformed plants carrying the insert (*PcGA20 ox* or *nptII* gene sequence) at the T₀ generation were obtained. Regenerated plants were successfully grown into mature fertile plants producing seeds. In the transformation procedure, a combination of different previously reported media for various crops have been successfully applied for embryogenic callus induction, *Agrobacterium* inoculation and co-cultivation and plant regeneration. The success in embryogenic callus induction using less than 1-week old zygotic immature embryos explants for regeneration into shoots was dependent on the use of intermediate size embryos. It was further found that the antibiotic geneticin (G418) fully controlled shoot growth from mature *E. tef* embryos which also requires further optimization for *E. tef* callus.

Molecular assessment of the transgene *PcGA2ox* has been based on results of previous studies where reduction in plant height in other cereals to be a key agronomic feature to limit lodging (Rajala, 2003; Rademacher, 2000). Results in this study showed only a putative T₀ transformants having a positive PCR results for the transgene. Selected putatively transformed T₀ plants were characterized further growing seeds (T₁ progeny) for genotype

and phenotype and inconsistent results were obtained during PCR detection of the presence of the transgene. Selected semi-dwarf T₁ generation plants showed that the reduction in plant height significantly varied even among the semi-dwarf plants. The reduction in height was also associated with amounts of bioactive GA₁. On the other hand, the accumulation of GA₈ in the semi-dwarf plants was not proportional to the relative height differences or supposedly deactivation of GA₁. Such phenomenon was also observed in transformed *Solanum nigrum* over-expressing same GA inactivating gene, *PcGA2ox* transgene (Dijkstra *et al.*, 2008). Deficiency through GA deactivation decreases height in rice (Lo *et al.*, 2008) with increasing yield (Ookawa *et al.*, 2010). However, due to the ambiguity of the PCR result, therefore, these plants have to be further characterized to show if *GA2ox* transgene expression such as *GA2ox* transcript abundance in the stem tissue is always associated with reduction in plant height to exclude the possibility of somaclonal variation induced in the tissue culture process using the auxin 2, 4-D (Banerjee *et al.*, 1985) and not due to stable transgene integration.

The fourth new aspect of this study was the successful isolation three *GA20ox* homologous genes from *E. tef*, involved in GA biosynthesis. Additionally, the *Rht* (*Reduced height*) and *Eui* (*Elongation Uppermost Internode*) genes involved in GA biosynthesis and a Cytochrome P450 monooxygenase gene in brassinosteroid deactivation were either fully or partially isolated and cloned. All these genes are involved in plant height control. Genomic analysis identified four copies of *GA20ox* to exist in *E. tef*. The *EtGA20ox1* sequence was successfully expressed in a heterologous bacterial system and allowed converting the substrate GA₁₂ to products GA₉, GA₁₅ and GA₂₄. Further, in *E. tef* *EtGA20ox1b* was the functional equivalent to the rice *sd-1* gene. Alignment and phylogenetic relationship of the full coding region of putative *E. tef* *GA2ox1* and partial putative *GA2ox1b* and *EtGA20ox2* sequences showed high identity scores with orthologous genes from *S. bicolor*, *Z. mays*, *O. sativa* and *L. perenne*.

This gene is expressed with highest transcriptional abundance in the uppermost internodes followed by node tissues and any mutation in this *E. tef* gene might specifically control plant height. However, a further study is required to confirm any functional similarity between *EtGA20ox1b* and *EtGA20ox1* in different plant tissues.

A fifth new aspect in this study was the potential usefulness of *E. tef* seed mutagenesis in producing a semi-dwarfed phenotype using variety DZ-Cr-37, which is a fairly tall modern cultivar. However, mutants combining reduced culm length and still good yield were not identified among the mutants screened. Except for semi-dwarf mutant line GA-10, which had a reasonable yield, low yield in tested dwarfed mutant lines was always associated with weak and infertile panicle development possibly due to undesirable random genetic changes not associated with height control. GA-10 also had a significantly higher diameter in most internodes which could directly contribute to the stiffness of stem and lodging resistance. This line also showed normal tillering and culm and panicle biomass and should therefore be tested further. Since all mutants were insensitive to GA application, the mutation in GA-10 might possibly be due to a change in GA signalling genes. Further analysis is therefore required if plants of this line harbour a GA signalling mutation. Also, crossing experiments with tall varieties are also required to verify and predict agronomic significance of line G-10 for lodging resistance.

Overall, support of the original hypothesis that regulation of the GA amount in *E. tef* will change pheno-morphic and also agronomic characteristics that would affect lodging and further decoupling plant height from yield was found in this study. GA inhibition or deactivation in *E. tef* produced a semi-dwarf phenotype without changing panicle development and grain yield except under conditions of severe GA inhibition where panicle

growth was affected and grain yield was reduced. Other pheno-morphic features or plant stand structures, such as diameter of basal internodes, the weak tapering (acropetally increasing stem diameter) and panicle bearing tillers in *E. tef* plant were not improved by reducing the endogenous GA amount.

Based on the findings of this study, any future following-up study might investigate stem tissue specific down-regulation of height controlling genes such as down-regulation of the already cloned *EtGA201a* in *E. tef* through a non-transgenic approach such as mutagenesis and TILLING or through RNAi technology. Panicle elongation was unaffected by GA inhibition, a future lodging-resistant improvement in *E. tef* also has to focus on obtaining compact type panicles in a dwarf or semi-dwarf phenotype background. Moreover, it is essential to further consider other potential threats in the future lodging resistant ideotype development in *E. tef* such as the narrow stem base problem (weak stem -basal to root-collar transition) that render *E. tef* plants weak in anchorage strength. Therefore, further study is required to understand mechanisms involved in stem-base diameter regulation (with emphasis on basal internodes), since the presumed weak transition from stem-base to the root-collar (supposedly low for *E. tef*) appears not to be regulated by GA. Generally based on the analyzed and implicated *E.tef* pheno-morphic/architectural traits in relation to lodging, the need for a more comprehensive intervention through gene regulation is clear and need to combine traits for a short stature, thicker basal-diameter and shorter panicle form in the lodging resistance *E. tef* ideotype development. The prime target genes related to stem height control that have already been cloned in this study can now be used to employ a non-transgenic approach using mutagenesis and TILLING techniques to induce mutation and select semi-dwarf genotypes in *E. tef* as an immediate future intervention strategy.