Manipulation of gibberellin biosynthesis for the control of plant height in *Eragrostis tef* for lodging resistance

By

ENDALE GEBRE KEDISSO

Thesis submitted in partial fulfilment of the requirements for the degree

PHILOSOPHIAE DOCTOR

Forestry and Agricultural Biotechnology Institute (FABI)

Department of Plant Science

In the

Faculty of Natural and Agricultural Sciences

University of Pretoria

SUPERVISORS: PROF. KARL KUNERT
DR. URTE SCHLÜTER

May 2012

© University of Pretoria
DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

Date: May 2012

Signed__________________________________

Endale Gebre Kedisso
Forestry and Agricultural Biotechnology Institute (FABI)
Department of Plant Science, University of Pretoria

This thesis has been submitted for examination with my approval as the University Supervisor.

Date _________________________________

Signed________________________________

PROF. KARL KUNERT
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>vii</td>
</tr>
<tr>
<td>THESIS COMPOSITION</td>
<td>ix</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>xi</td>
</tr>
<tr>
<td>ABBREVIATIONS AND SYMBOLS</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xxiii</td>
</tr>
</tbody>
</table>

CHAPTER ONE: INTRODUCTION

1.1. The problem of lodging  2
1.2. Plant architecture and lodging  4
1.3. Genetic control of lodging resistance  6
    1.3.1. GA genes and lodging  6
    1.3.2. Manipulation of plant height using GA genes  10
1.4. Brassinosteroid (BRs) genes and lodging  13
1.5. Induced mutation  14
1.6. Plant growth regulators for plant height control  17
1.7. Lodging in *E. tef*  21
    1.7.1. *E. tef* growth  21
    1.7.2. Pheno-morphic features related to lodging  22
    1.7.3. *E. tef* breeding for lodging resistance  24
1.8. Working hypothesis and aim of study  26
CHAPTER TWO: CONTROLLING PLANT HEIGHT AND LODGING IN TEF (Eragrostis tef) USING GIBBERELLIN BIOSYNTHESIS INHIBITORS

2.1. Abstract 28
2.2. Introduction 29
2.3. Materials and methods 31
  2.3.1. Plant material 31
  2.3.2. Plant growth 31
  2.3.3. Plant growth regulators (PGRs) treatment 32
  2.3.4. Growth measurement 33
  2.3.5. Analysis of endogenous GA content 33
  2.3.6. Data analysis 34
2.4. Results 35
  2.4.1. Experiment I 35
    2.4.1.1. Analysis of endogenous GA content 37
  2.4.2. Experiment II 39
    2.4.2.1. Culm and panicle growth 39
    2.4.2.2. Internode growth 40
    2.4.2.3. Tillering and above ground biomass yield 43
2.5. Discussion 51

CHAPTER THREE: TRANSFORMATION OF TEF (Eragrostis tef)

3.1 Abstract 58
3.2 Introduction 60
3.3 Materials and methods 61
  3.3.1 Preparation of planting material and culture 61
  3.3.2 Immature embryo isolation, callus induction and culture growth 62
  3.3.3 GA20x and nptII marker gene plasmids 63
  3.3.4 Agrobacterium culture, inoculation and co-cultivation 65
  3.3.5 Plant regeneration 66
CHAPTER FOUR: ISOLATION, CHARACTERIZATION AND EXPRESSION OF GA GENES WITH PARTICULAR EMPHASIS ON GA20ox IN TEF (Eragrostis tef)

4.1 Abstract 89
4.2 Introduction 90
4.3 Materials and methods 92
   4.3.1 Plant material and plant growth 92
   4.3.2 Genomic DNA isolation 92
   4.3.3 Gene identification and isolation 93
   4.3.4 Isolation of complete *E. tef* GA20ox coding region 95
   4.3.5 Cloning and sequencing of PCR DNA products 97
   4.3.6 DNA sequence analysis and phylogentic analysis 98
   4.3.7 RNA isolation and cDNA synthesis 99
4.3.8 Isolation of the Reduced height (Rht) and other E. tef genes
4.3.9 GA20-oxidase expression in E. tef
4.3.10 Expression of E. tef GA20-oxidase1 (EtGA20ox1) in E. coli
4.3.11 HPLC analysis
4.3.12 Southern blot analysis

4.4 Results
4.4.1 Isolation of GA genes from E. tef
4.4.2 Putative E. tef GA20ox isolation and cloning
4.4.3 EtGA20ox copy number
4.4.4 GA20ox expression in E. tef
4.4.5 In vitro enzymatic activity of GA20ox in a heterologous system
4.4.6 Isolation of the Rht and other E. tef genes

4.5 Discussion

CHAPTER FIVE: EVALUAUTION AND ANALYSIS OF MUTANT TEF
(Eragrostis tef) LINES FOR DWARFISM FOR LODGING RESISTANCE

5.1 Abstract
5.2 Introduction
5.3 Materials and methods
5.3.1 Plant material
5.3.2 Plant growth and GA treatment
5.3.2.1 Growth measurement
5.3.2.2 Data analysis
5.4 Results
5.4.1 Culm height, internode length and diameter
5.4.2 Panicle length, tillering, biomass and yield
5.5 Discussion

CHAPTER SIX: GENERAL DISCUSSION AND FUTURE PERSPECTIVE
REFERENCES
APPENDIX
ABSTRACT

Manipulation of gibberellin biosynthesis for the control of plant height in *Eragrostis tef* for lodging resistance

Endale Gebre Kedisso
Plant Science Department, Forestry and Agricultural Biotechnology Institute (FABI), 74 Lunnon Road, Hillcrest 0002, University of Pretoria, South Africa

Supervisor: **Prof. Karl Kunert**
Plant Science Department, Forestry and Agricultural Biotechnology Institute (FABI), 74 Lunnon Road, Hillcrest 0002, University of Pretoria, South Africa

Co-Supervisor: **Dr. Urte Schlüter**
Plant Science Department, Forestry and Agricultural Biotechnology Institute (FABI), 74 Lunnon Road, Hillcrest 0002, University of Pretoria, South Africa

Lodging is a key agronomic problem in *E. tef*. due to morpho-physiological features, such tall and slender phenotype of the plant. Gibberellins metabolic genes are key targets in the control of plant height. Plant growth regulators (PGRs) that inhibit GA biosynthesis are used to shorten stem length thereby increasing lodging resistance. *E. tef* responded to treatment with PGRs such as GA, chlormequat chloride (CCC) and paclobutrazol (PBZ). Both PGRs reduced *E. tef* plant height but CCC treatment did not affect grain yield. Stem diameter was not affected by PGR treatment and also not the poor tapering (acropitally increasing diameter).

Putatively transformed *E. tef* plants carrying a bean GA 2-oxidase (*PcGA2ox*) coding sequence were further produced via embryogenic callus after Agrobacterium-mediated transformation and plants were successfully grown into mature fertile plants. Eight putative transformed plants were finally generated carrying the insert (*PcGA20ox* or *nptII* gene...
sequence) at the T₀ generation. Constitutive expression of the GA 2-oxidase (PcGA2ox) coding sequence in E. tef resulted in phenotypic changes such as reduction in culm height, change in biomass, reduction in amount of GA in putative transformant semi-dwarf plants. The challenges found in the transgene detection in the T₁ generation has been highlighted. Pheno-morphic changes occurred with little or no effect on yield.

Genes involved in height control (orthologs to the rice sd-1 gene) and signaling (Rht) in E. tef were also identified and characterized. Activity of the protein for the putative rice sd-1 orthologs was further confirmed by heterologous expression. The three putative sequences in E. tef were named EtGA20ox1a, EtGA20ox1b and EtGA20ox2. Expression analysis showed that EtGA20ox2 were much less transcribed compared to the others and EtGA20ox1b could be the functional equivalent to the rice sd-1 (OsGA20ox2) gene in E. tef.

Further, E. tef mutants with a semi-dwarf phenotype could be developed through mutagenesis and TILLING. However, regardless of height, grain yield was severely reduced in all mutants except in the semi-dwarf mutant GA-10. This line also had significantly higher diameter in most internodes which might contribute to the stiffness of stem. G-10 is therefore a promising line for further investigations.
Thesis composition

Chapter 1 of this thesis provides a summary of the lodging problem in cereals and alternative methods (chemical and genetic approaches) used to control lodging as well as the traits involved. An up-to-date review of the lodging problem in *E. tef* including phenomorphic features relating to lodging and experiences in other crops as well as in the “green revolution” are outlined. Approaches solving the lodging problem and genes that play a key role in plant architecture modification in cereal crops for improving lodging resistance are discussed. The rationale, aim, and objectives for carrying out this study are further outlined at the end of the introduction. In Chapter 2, results obtained from treatment of *E. tef* plants with GA biosynthesis inhibitors in controlling plant height are presented. This includes treatment with GA$_3$, CCC and Paclobutrazol and changes in plant height and other phenomorphic and agronomical features due to PGR treatment are reported. Chapter 3 reports about transforming *E. tef* plants using immature somatic embryos via embryogenic callus for Agrobacterium-mediated transformation. Successful regeneration of putative transformed plants after a transformation procedure using combinations of different media is outlined. Moreover, characterization of plants over-expressing GA2 oxidase from *Phaseolus coccineus* (*PcGA2ox1*) for inducing dwarfism is presented and results of characterizing putatively transformed T0 generation plants regarding their morpho-physiological features and expression of a semi-dwarf phenotype with reduced height are reported. The inconsistent PCR results at T1 and the possibility that any found differences could also be due to somaclonal variations owing to the relatively higher rate of auxin applied is indicated. Chapter 4 outlines the identification, and characterization of height-controlling genes. This includes the rice homologous *SD-1* in *E. tef*, the wheat *Rht* orthologue and two Cytochrome P450 monooxygenase genes (*Eui* and Brassinosteroid deactivation genes). Also an activity
assay through heterologus expression of EtGA20ox1 in E. coli and specific tissue expression of the three EtGA20ox homologs as well as copy number of these genes in the E. tef genome are presented. In Chapter 5, data on phenotype (plant stature) characterization is outlined for selected mutant E. tef lines developed through mutagenesis and TILLING to generate sufficient variability for semi-dwarfism in E. tef for lodging resistance. Morphological and physiological attributes and agronomic relevance of these mutant lines are described in terms of plant height reduction, tillering, biomass and yield. Chapter 6 finally summarizes the findings and relevant information developed in this PhD study. It also outlines the salient features that need to be considered further in a lodging-resistant E. tef ideotype. This is followed by the list of citations (References) used in this dissertation. The Appendix provides further sequence results (nucleotides and translated amino acid) from the gene cloning and characterization study, alignment and phylogenetic analysis of E. tef sequences with different species.
ACKNOWLEDGEMENT

First of all, I thank The Lord Almighty for His abundant love and grace, his inspiration providing me with the wisdom needed to pursue science with enthusiasm. PRAISE YOUR NAME O LORD!

My deepest gratitude goes to my mother, Askale Nida and my father Gebre Kedisso for their love and caring implanting in me so much good things. I feel proud of my elder and younger brothers and sisters for their constant prayers and the pure love we share.

I express my deep gratitude to my supervisor Prof. Karl Kunert for his follow up, guidance and critical help and inputs encouraging me all throughout my study period. Thank you also for creating opportunities to attending conferences and research visit to Rothamsted Research in UK and for creating linkages to research groups in different countries. All this have considerably facilitated my study and contributed to acquiring skills. Thank you for your great concern, patience and kindness.

My earnest gratitude also goes to my co-supervisor Dr. Urte Schlüter for her very valuable inputs, advices and encouragement during the course of my study. Thank you also for the help creating opportunity linking my research to groups at Rothamsted, UK which has been very valuable to my study and for successfully finishing my experiments.

I am also very grateful to Prof. Peter Hedden for the supervision and guidance during my six month stay at Rothamsted Research working in his lab. It was a very valuable time I spent. I am also indebted to Dr. Simon Volghur for his kind assistance in molecular analysis and
technical help during and after my visit. I also would like to thank colleagues at Molecular biology lab and Experimental Greenhouse at Rothamsted Research for their kind support.

I also am very grateful to Prof. Maryon J Mayer, Head of the Plant Science Department, and Rene Stewart Executive Secretary of the department for the positive and kind help and encouragement in times of need during my study. I also thank the Forestry and Agricultural Biotechnology Institute (FABI) management and team for the very good working environment I enjoyed to be part during my study period.

I would like to thank Dr. Solomon Assefa, Director EIAR, for his support and positive consideration of my extension request and Dr. Adefris T/Wold who shared with me the idea of working on tef as well as for his kind help and encouragement during the study period. I would also like to thank Dr. Likyelesh Gugssa for the very valuable help providing me with technical information on tef transformation and other materials and Dr. Kebebew Assefa providing me with valuable publications on tef at the beginning of my study. I am also very grateful to Dr. Zerihun Tadelle for his cooperation to work on some of his mutagenized lines for my experimentation.

An earnest thank you to my entire lab mates in SA, Berhanu, Rosita, Priyen, Ryhandr, Stephan, Magdeleen, Celia, Tsholofelo, Kutzai and Abigel for the nice environment of interactions we kept alive in the lab, for the concern and help during the long hours of work together in those years. I am grateful to my FABI friends for the kindness during my study period. I am particularly very thankful to the unreserved help I received from Tuan, Magriet and Markus.
I want to sincerely thank my Ethiopian friends studying at UP for the warm friendship and good social environment we enjoyed together. I thank Ethiopian students who lived in Tuks Dorp, UP namely Berhanu, Dr. Wubetu, Dawit, Dr. Legesse, Hiywot, Dr. Temesgen, Ato Habtamu, Dr. Yemaneh, Meheretu, Yebeltal and others for their friendship and support. After moving to Sunnyside, I missed a lot the football game we established and the small evening running we used to do. I am also very thankful particularly to Yohannes (John), Dr. Abayneh, Tedlaye and their families for the unforgettable time my wife and I had together with them. I also thank Kebron Church leaders and Christian friends in the Ethiopian Christian Fellowship for the great time we had together that made our stay in Pretoria such a blessing.

This PhD research work was sponsored by the Ethiopian Institute of Agricultural Research (EIAR), Rural Capacity Building Project (RCBP) and partly by the Rothamsted International (RI) and was carried out at the Food and Agricultural Biotechnology Institute (FABI), Department of Plant Sciences, University of Pretoria and at Rothamsted Research, UK. I am very grateful to the above institutions that constituted the financial backbone for my study.
DEDICATION

I dedicate this thesis to my wife Meseret Worku, whose understanding, love, kindness and help enabled me to pursue the work to the end; and to my parents, brothers and sisters who occupy such a special place in my heart for their love and inspiration uplifting my spirit through their prayers.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>Percentage</td>
</tr>
<tr>
<td>µg</td>
<td>Microgram</td>
</tr>
<tr>
<td>µL</td>
<td>Microlitre</td>
</tr>
<tr>
<td>2-ODD</td>
<td>2-Oxoglutarate dependent dioxygenase</td>
</tr>
<tr>
<td>bp</td>
<td>Base pair</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>CCC</td>
<td>Chlormequate chloride</td>
</tr>
<tr>
<td>CCM</td>
<td>Co-cultivation medium</td>
</tr>
<tr>
<td>CaMV</td>
<td>Cauliflower mosaic virus</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>cDNA comp.</td>
<td>Complimentary DNA</td>
</tr>
<tr>
<td>CPS</td>
<td>ent-copalyl diphosphate synthase</td>
</tr>
<tr>
<td>Ct</td>
<td>Cycle threshold</td>
</tr>
<tr>
<td>CTAB</td>
<td>hexadecyl-trimethyl-azanium bromide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DNase</td>
<td>Deoxyribonuclease</td>
</tr>
<tr>
<td>dNTP</td>
<td>deoxy nucleotide triphosphate</td>
</tr>
<tr>
<td>DZ</td>
<td>Debre Zeit</td>
</tr>
<tr>
<td>E. coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediamine tetra acetic acid</td>
</tr>
<tr>
<td>EMS</td>
<td>ethylmethanesulphonate</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic reticulum</td>
</tr>
<tr>
<td>EUI</td>
<td>elongated uppermost internode</td>
</tr>
<tr>
<td>g</td>
<td>Grams</td>
</tr>
<tr>
<td>GA&lt;sub&gt;n&lt;/sub&gt;</td>
<td>Gibberellin A&lt;sub&gt;n&lt;/sub&gt;</td>
</tr>
<tr>
<td>GA-ox</td>
<td>Gibberellin -oxidase</td>
</tr>
<tr>
<td>GA 2-ox</td>
<td>Gibberellin 2-oxidase</td>
</tr>
<tr>
<td>GAI</td>
<td>Gibberellin insensitive</td>
</tr>
<tr>
<td>gDNA</td>
<td>genomic deoxyribonucleic acid</td>
</tr>
<tr>
<td>GGPP</td>
<td>geranyl-geranyl diphosphate</td>
</tr>
<tr>
<td>GID</td>
<td>Gibberellin insensitive dwarf</td>
</tr>
<tr>
<td>GUS</td>
<td>β-Glucuronidase</td>
</tr>
<tr>
<td>h</td>
<td>hours</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>Water</td>
</tr>
<tr>
<td>IE</td>
<td>Immature embryo</td>
</tr>
<tr>
<td>IPTG</td>
<td>Isopropyl-β -d-thiogalactopyranoside</td>
</tr>
<tr>
<td>KAO</td>
<td>ent-kaurenoic acid oxidase</td>
</tr>
<tr>
<td>KO</td>
<td>ent-kaurene oxidase</td>
</tr>
<tr>
<td>KS</td>
<td>ent-kaurene synthase</td>
</tr>
<tr>
<td>L</td>
<td>Litre</td>
</tr>
<tr>
<td>LB</td>
<td>Luria broth</td>
</tr>
<tr>
<td>LCM</td>
<td>Laser capture microdissection</td>
</tr>
</tbody>
</table>
M  Molar
min  minute
mL  Millilitres
mM  Millimolar
NaAC  Sodium acetate
NaCl  Sodium chloride
NaOH  Sodium hydroxide
ng  Nanogram
NTC  No-target control
°C  Degree Celcius
ORF  Open reading frame
PBZ  Paclobutrazol
PcGA2ox  Phaseolous coccinus GA 2-oxidase
PCR  Polymerase chain reaction
PPFR  Photosynthetic photonflux rate
qRT-PCR  quantitative reverse transcriptase polymerase chain reaction
RACE  Random Amplified cDNA Ends
RGA  repressor of ga1-3
RHT  Reduced height
RNA  Ribonucleic acid
RNAase  ribonuclease
rpm  Revolutions per minutes
s  Second
sd H2O  Sterile distilled water
SD1  semi-dwarf 1
SDS  Sodium dodecyl sulphate
SLN  Slender
SNP  Single Nucleotide Polymorphism
TILLING  Targeting induced local lesions in genomes
UV  ultra violet
v/v  volume per unit volume
w/v  weight per unit volume
wk  week (s)
LIST OF FIGURES

Figure 1.1
Phenotypic variations for allelic diversity for semi-dwarfing traits in the green revolution genes from wheat (Rht) and rice (sd-1) 8

Figure 1.2
Partial protein sequence encoding Rht-B1a and Rht-B1b loci from wheat showing the few amino acid internal deletion that contributed to wheat semi-dwarfism 8

Figure 1.3
Simplified scheme of the GA biosynthesis steps and points of inhibition by growth retardants 12

Figure 1.4
Simplified scheme of the gibberellins (GA) biosynthesis pathways and deactivation by GA 2-oxidase in plants 19

Figure 1.5
E. tef plant stand in the field at (A) grain filling and (B) at maturity when almost all plants lodged 23

Figure 2.1
Growth (plant height) response to exogenous application of GA and CCC after six weeks of Gea Lammie and DZ-01-196 plants grown in greenhouse. 35

Figure 2.2
Tillering, fresh and dry weight responses of six weeks old DZ-01-196 seedlings to CCC treatment 36
Figure 2.3
Effect of foliar applied CCC on fresh and dry weight of six weeks old seedlings

Figure 2.4
Effect of CCC (100mM) and PBZ (100µM) on plant height near plant maturity

Figure 2.5
Comparison of plant height and panicle growth at plant maturity as affected by PGRs

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

Figure 2.7
Effect of different concentrations of CCC and PBZ on primary (1º) and secondary (2º) tiller grain yield per plant in comparison to the untreated control

Figure 2.8
Comparison of panicle elongation with PBZ treatments in proportion to culm reduction in DZ-01-196
Figure 3.1
Construction of plasmid pGPTV-Kan harbouring \textit{Pc2ox1} and the triple 35S CaMV promoter sequence

Figure 3.2
\textit{E. tef} shoot regeneration using immature embryo from young emerging panicle as explants

Figure 3.3
PCR amplification of \textit{PcGA2 ox} and \textit{nptII} sequences from putative transformed plants of T\textsubscript{0} and T\textsubscript{1} generation

Figure 3.4
Culm and panicle height (cm) and number of tillers per plant of putatively transformed dwarf \textit{E. tef} plants

Figure 3.5
Selected \textit{E. tef} dwarf (18) and semi-dwarf T\textsubscript{1} generation plants (19(3), 19(2), 28 and 10)

Figure 3.6
Seed weight of primary and secondary panicles of dwarf \textit{E. tef} lines and a control

Figure 3.7
Comparison of endogenous GA levels in the GA biosynthetic pathway between dwarf and controls plants

Figure 4.1
Nucleotide sequence alignment of three putative tef \textit{GA20ox} sequences
**Figure 4.2**
Derived nucleotide sequence alignment of the two tef $GA20oxs$ ($GA20ox1$ and $GA20ox1b$) sequences with sequences from other cereals  

**Figure 4.3**
Derived nucleotide sequence alignment of tef $GA20ox2$ sequence with orthologous $GA20ox$ gene sequences from other cereals and Arabidopsis  

**Figure 4.4**
Molecular phylogenetic analysis of the homologous of $E. tef$ $GA20ox$ sequences ($GA20ox1$, $GA20ox1b$ and $GA20ox2$)  

**Figure 4.5**
Detection of EtGA20 ox gene copies in the $E. tef$ genome after restriction enzyme digest using Southern Blot  

**Figure 4.6**
Relative expression of $GA20ox1$ in two $E. tef$ genotypes DZ-01-196 and Gea Lammie in different plant tissues and growing stages  

**Figure 4.7**
Semi quantitative RT-PCR expression analysis of three EtGA20ox genes in various plant tissues in variety DZ-01-196  

**Figure 4.8**
Radiochromatograms after HPLC of $E. tef$ $GA20ox1$ activity products after incubation with a $^{14}$C-labeled GA$_{12}$ as a substrate
Figure 4.9
Amino acid sequence alignment of the putative tef Rht sequences with orthologous amino acid sequences from other species

Figure 4.10
Molecular Phylogenetic analysis of E. tef Rht peptide sequences by Maximum Likelihood method

Figure 5.1
Culm and panicle length and seed weight (gm) in plants of different mutant lines and the control

Figure 5.2
Culm length of plants of different mutant lines and wild type plants with and without GA treatment

Figure 5.3 Effect of GA application on peduncle and panicle length of mutant lines and the control

Figure A.1
E. tef GA20ox1 full coding region and deduced amino acid residues of E. tef GA20ox1 sequence with conserved domains.

Figure A.2
Homologous sequences of E. tef GA20ox1a and GA20ox2 partial coding region, and deduced amino acid residues

Figure A.3
Partial coding region of the Elongated Uppermost Intenode (EUI) gene in E.tef

Figure A.4
Derived nucleotide sequence alignment of the putative tef *Uppermost Elongated Internode (EUI)* with ortholog sequences from other cereals 181

**Figure A.5**

Molecular phylogenetic analysis of the rice *EUI* ortholog gene in *E. tef* 182

**Figure A.6**

Full coding region of the *E. tef* *RHT* gene near full coding region (1397 bp) and deduced amino acid residues with characteristic DELLA, VHYNP and VHVVD domains 183

**Figure A.7**

Putative *E. tef* brassinosteroid deactivation related Cytochrome P450 monooxygenase gene partial nucleotide sequence 184

**Figure A.8**

Amino acid sequence alignment of the putative brassinosteroid deactivating gene sequences from *E. tef* with amino acid sequences from other species 185

**Figure A.9**

Molecular Phylogenetic analysis of putative *E. tef* brassinosteroid deactivation gene sequence 187
## LIST OF TABLES

**Table 1.1**  
Semi-dwarf sources in wheat induced by chemical or physical mutagens  

**Table 2.1**  
Quantification of GA intermediates and bioactive forms available in two *E. tef* plant varieties at stem elongation  

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

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

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

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

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

**Table 3.1**
Media used for induction of embryogenic callus, co-cultivation, selection and regeneration of transformed *E. tef* shoots

**Table 3.2**
Composition of the K99EM medium used for embryogenic callus induction from *E. tef* immature embryos

**Table 3.3**
Ionic composition of the K4NB regeneration media used for tef immature embryo cultures

**Table 3.4**
Survival of non-transformed *E. tef* seedlings derived from mature embryo on antibiotic (G418)-containing selection medium

**Table 3.5**
Above ground biomass of putatively transformed dwarf tDZ-01-196 *E. tef* plants

**Table 4.1**
Primers used in PCR to amplify GA20ox gene fragments using *E. tef* genomic DNA

**Table 4.2**
Primers used or GA20ox sequence RACE- PCR amplification

**Table 4.3**
Primers used in quantitative and semi-quantitative PCR amplification of GA20ox sequences

**Table 5.1**
Peduncle length, internode number, number of tillers, culm and panicle dry weight per plant of mutant *E. tef* lines

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

Table 5.3
Peduncle length, internode number, number of tillers, culm and panicle dry weight per plant of mutagenized *E. tef* lines

Table 5.4
Internode diameter of mutant lines in response to exogenous GA treatment

Table A.1
Primers used for PCR amplification of gene fragments of *Rht*, *Eui* and *BR* gene sequences using *E. tef* genomic DNA

Table A.2
*Rht* primers used in RACE-PCR