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

By

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DECLARATION

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

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

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ABSTRACT

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

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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 (*PcGA20 ox* or *nptII* gene

sequence) at the T_0 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 dectection 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* (*OsGA20ox*2) 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 pheno-morphic 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 pheno-morphic 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 phylogentic analysis of *E. tef* sequences with different species.

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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.

ABBREVIATIONS AND SYMBOLS

%	Percentage
µg	Microgram
µL	Microlitre
2-ODD	2-Oxoglutarate dependent dioxygenase
bp	Base pair
BSA	Bovine serum albumin
CCC	Chlormequate chloride
CCM	Co-cultivation medium
CaMV	Cauliflower mosaic virus
cDNA	complementary deoxyribonucleic acid
cDNA	Complimentary DNA
CPS	*ent*-copalyl diphosphate synthase
Ct	Cycle threshold
CTAB	hexadecyl-trimethyl-azanium bromide
DMSO	dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	deoxy nucleotide triphosphate
DZ	Debre Zeit
E. coli	Escherichia coli
EDTA	Ethylenediamine tetra acetic acid
EMS	ethylmethanesulphonate
ER	Endoplasmic reticulum
EUI	elongated uppermost internode
g	Grams
GA_n	Gibberellin A_n
GA-ox	Gibberellin -oxidase
GA 2-ox	Gibberellin 2-oxidase
GAI	Gibberellin insensitive
gDNA	genomic deoxyribonucleic acid
GGPP	geranyl-geranyl diphosphate
GID	Gibberellin insensitive dwarf
GUS	β-Glucuronidase
h	hours
H_2O	Water
IE	Immature embryo
IPTG	Isopropyl-β -d-thiogalactopyranoside
KAO	*ent*-kaurenoic acid oxidase
KO	*ent*-kaurene oxidase
KS	*ent*-kaurene synthase
L	Litre
LB	Luria broth
LCM	Laser capture microdissection

M	Molar
min	minute
mL	Millilitres
mM	Millimolar
NaAC	Sodium acetate
NaCl	Sodium chloride
NaOH	Sodium hydroxide
ng	Nanogram
NTC	No-target control
^{o}C	Degree Celcius
ORF	Open reading frame
PBZ	Paclobutrazol
PcGA2ox	*Phaseolous cuccinous* 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 H_2O	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)

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