



CHAPTER 1

INTRODUCTION

1.1 The problem of lodging

Lodging, as a mechanical plant stress, is a complex interaction of the plant with the environment including factors such as light, wind, temperature, rain, topography, soil type, nutrition, plant density and diseases (Berry *et al.*, 2004) and is frequently associated with plant pheno-morphic characteristics (Pinthus, 1973). It is a process by which the shoot of a small grained cereal is displaced from their vertical stance. The standing strength of the stem, or the root, to hold up the shoot leverage is threatened by adverse weather conditions. Lodging is also variety (genotype)-dependent and a tall, weak-stemmed variety is more prone to lodge than a semi-dwarf variety with stiffer stem. It occurs in the form of stem lodging or failure of the anchorage system (root lodging) (Pinthus, 1973; Thomas, 1982; Crook and Ennos, 1993; Graham, 1983 and Delden *et al.*, 2010).

Lodging, causes a direct yield loss due to falling-over of the plant and also an indirect loss due to limiting optimum use of nitrogen fertilizer. Problem of reduced lignifications of the stem also reduces stem strength following high nitrogen fertilizer use, which is counter-productive, and any increase in grain biomass is offset by increased lodging (Crook & Ennos 1994). Lodging also restricts mechanical seed harvesting and reduces seed quality. Furthermore lodging can also limit efficient light interception; reduce translocation and assimilation, increase respiration and chlorosis thus affecting growth and development processes in the plant system (Berry *et al.*, 2004). Because of the significance in the global agricultural economy, wheat and rice were the two main field crops in which it was desirable to substantially reduce significant yield losses due to lodging of tall, wild-type genotypes. Applying nitrogen fertilizer was not successful, since it aggravated the lodging problem. Shorter, sturdier, semi-dwarf varieties were developed that have been far more resistant to

lodging and also exhibited an unexpected benefit of improved assimilation into reproductive organs due to pleiotropic effects (Hanson *et al.*, 1982). Thus an essential trait of the higher-yielding varieties was reduced (dwarf) stature that enabled large increases in yield to be obtained.

Therefore, in many cereal crops, plant height has been the main target for improvement of lodging resistance incorporating the trait in modern cultivars. For instance the semi-dwarf wheat variety from Japan (Norin 10) contains the most important dwarfing genes that confer reduced height trait used today in wheat. This variety originated from a cross between the native Japanese dwarf variety Daruma with two American wheat varieties, Fultz and Turkey Red. The genes conferring semi-dwarfing traits are for culm shortening with a semi-dominant gain-of-function mutation in the *REDUCED HEIGHT (RHT)* homologous genes in wheat (Gale and Marhsall, 1976). These genes were introduced into many other varieties grown in many parts of the world (Silverstone *et al.*, 2001) and 70% of modern wheat varieties carry at least one of the dwarfing genes (Hedden, 2003). In rice, the recessive semi-dwarf (*sd-1*) gene has been used to reduce plant height and considerably improved lodging resistance. The mutated *sd-1* in rice existed in semi-dwarf native varieties. In the 1960s the dwarfing trait was incorporated into improved rice lines to develop a semi-dwarf phenotype in rice (Ashikari *et al.*, 2002). In barley, the value of short variety was understood earlier and shorter barley varieties such as ‘Valticky’ that originated from local landraces was widely grown replacing taller varieties in Moravia at the beginning of the twentieth century (Bouma and Ohnoutka 1991).

Generally, taking in to account wheat and rice, mutations in the *Rht* and *sd-1* genes have been among the main factors responsible for higher yields obtained in the “green revolution”

(Yamaguchi, 2008; Kashiwagi and Ishimaru, 2004; Hedden, 2003). As a result the average yield in wheat has increased from 2.2 t ha⁻¹ to 6.0 t ha⁻¹, whereas in rice yield has increased due to introduction of semi-dwarf varieties (Berry *et al.*, 2004) from 1.5 t ha⁻¹ to 4.2 t ha⁻¹. Thus the identification and subsequent introgression of stem height controlling genes was considered the principal factors for reduced lodging allowing higher amount of fertilizer use in wheat and rice during the 70s of the last century (Tong, 2007; Kashiwagi and Ishimaru, 2004; Berry *et al.*, 2004; Hedden, 2003).

1.2 Plant architecture and lodging

Plants architectures refers to the degree of branching, internodal elongation, and shoot determinacy (Wang and Li, 2008). The architecture of plants is linked to plant functions including efficient water transport, light interception, soil resource acquisition, and the maintenance of a mechanically stable structure. To optimize their functions plants keep building their architecture gradually in response to changes in environmental conditions (Sterck, 2005). When plants lose its optimum architecture orientation or vertical stance required for optimal function in a given set of environment, due to mechanical failure, conditions that promote plant growth is interrupted. A favourable environment is created for diseases increasing harvesting cost and yield loss. The culm structure, such as length, diameter, shape, composition, degree of branching, leaf arrangement and orientation, internodal elongation, and shoot (determinate or not), define the type of architecture of a crop. These factors determine the specific pheno-morphic and physio-morphic features and define the specific interaction the plant may have with environmental elements such as wind, rain, soil, nutrient and light (Berry *et al.*, 2004).

Lodging occurs when the stem strength, which depends on stem diameter and the composition and width of the stem wall, is insufficient to hold the shoot up against leverage. It also occurs due to failure of the anchorage system, which depends on the spread and depth of the root plate and the strength of surrounding soil, causing the root to have insufficient strength to hold the shoot up. Stem lodging takes place in the form of “buckling” of the lower internodes or the middle internodes called “brackling” (Thomas, 1982; Neenan and Spencer-Smith, 1975), or the breaking of the peduncle known as “necking” found in wheat, barley, oats (White, 1991). It can also occur in the form of root lodging (failure of anchorage system) which results in a permanent displacement of the stem found in wheat, barley, and oats and also in *E. tef* (Pinthus, 1973; Crook and Ennos, 1993; Graham, 1983 and Delden *et al.*, 2010). Berry *et al.* (2004) have further shown that root lodging could be predominant. Reports, however, have often been in favour of stem (straw) lodging indicating that misconception can occur about the reason of failure in lodged crops. In *E. tef* grown in sandy soils, root anchorage failure and insufficient stem strength have been found (Delden *et al.*, 2010). However, earlier studies with *E. tef* have shown that stem length thickness or diameter of basal internodes, panicle length and weight, and earliness to be important traits for lodging resistance (Berhe, 1981; Ketema, 1983; Mengesha *et al.* 1965; Hundera *et al.*, 1999; Asefa *et al.*, 2000 and Yu *et al.*, 2007).

Further, optimum fertilizer application for increasing grain yield promotes lodging due to increasing plant height. However, under conditions of high fertilizer (N) and moisture, varieties having a semi-dwarf stature are less prone to lodging. If semi-dwarf varieties are further have thick-straw, resistance to lodging is greatly improved.

1.3 Genetic control of lodging resistance

1.3.1 GA genes and lodging

The first indication that GAs were endogenous growth regulators in plants was reported in the 1950s, after study showed the height of dwarf pea and maize mutants were restored to normal by applying gibberellic acid (GA₃) (Hedden and Phillip, 2000). Among the many influences of GA genes on the plant growth and development, their ability to promote internodal elongation in a wide range of species that belong to the grass family has been of considerable agronomic importance (Taiz and Zeiger, 2006). Basically the importance of GA was demonstrated by the discovery of GA-deficient (reduced bioactive GA amounts) or GA-insensitive mutants in several species, including rice (Murakami, 1970), wheat (Maluszynski and Szarejko, 2005), maize (Phinney, 1956), and *Arabidopsis* (Koornneef and Van der Veen, 1980). These mutants had typically a dwarf or a semi-dwarf phenotype with a reduced bioactive GA amount in case of GA-deficient mutants or high bioactive GA concentrations in certain GA-insensitive dwarf mutants, such as *Rht3* wheat and *Dwarf-8* maize (Hedden and Kamiya, 1997) due to a negative feed-back regulation (Alvey and Harberd, 2005). The use of highly sensitive methods of physio-chemical analysis, such as gas chromatography and mass spectrometry (GC-MS), has shown that the GAs are large group of natural products, up to 126 different compounds currently known. However, based on the analysis of the GA-deficient mutants only few GAs have intrinsic biological activity and a hormonal actions (Hedden and Phillip, 2000).

Identification and isolation of the homologous *sd-1* (GA-deficient) and *Rht* (GA-insensitive) genes in *Arabidopsis* has further helped to understand the role of GA genes in plant height control. The *sd-1* gene encodes a GA biosynthetic enzyme, GA20 oxidase, and the rice genome carries four GA20- oxidase genes, *GA20ox1-4*. The *sd-1* corresponds to *GA20ox-2*, which is highly expressed in leaves and flowers (Yamaguchi, 2008). The enzymatic action of the two other oxidases is not well-known (Hedden *et al.*, 2002). GA 20-oxidase is a regulatory enzyme with multifunctional catalytic activity acting at several stages in the biosynthesis process and the oxidase is further a prime target in the genetic manipulation of the GA biosynthetic pathway (Hedden *et al.*, 1998). The function of *RHT* was only identified as a result of molecular genetic studies on the analogous gibberellic acid-insensitive (*ga-insensitive*) *gai* mutant of *Arabidopsis*. *GAI* encodes a GA response repressor gene in the GA response pathway which functions in the absence of GA (Peng *et al.*, 1997). The *Arabidopsis* *GAI* dwarf mutants have further been found to be orthologs of the maize dwarf (*D8*) genes (Fu *et al.*, 2001). Unlike *sd-1*, the phenotype of these dwarf mutants could not be restored to the wild-type by exogenous GA application due to a mutation in the GA response pathway (Taiz and Zeiger, 2006). Further studies revealed that *RHT* has multiple allelic variants resulting in variations of the *RHT* mutant (Figure 1.1). Three amino acid deletions and introduction of a stop codon at the N-terminus of its coding region further resulted in a semi-dwarf phenotype (Figure 1.2).

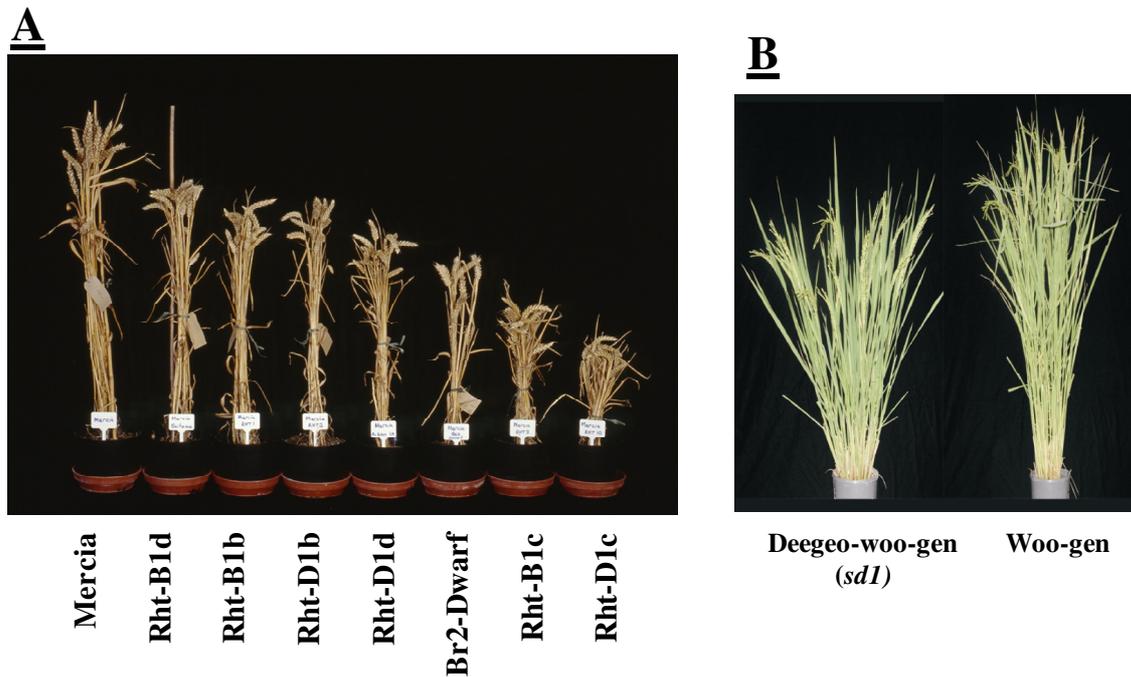


Figure 1.1 A) Phenotypic variations for allelic diversity for semi-dwarfing traits for the wheat *Rht* gene (except Br2-dwarf which is a brassinosteroid insensitive dwarf) (Peng *et al.* 1999 and Pearce *et al.* (unpublished)) and B) for the semi-dominant (*sd-1*) semi-dwarfing gene in rice (Monna *et al.* 2002, Sasaki *et al.* 2002 and Spielmeyer *et al.* 2002).

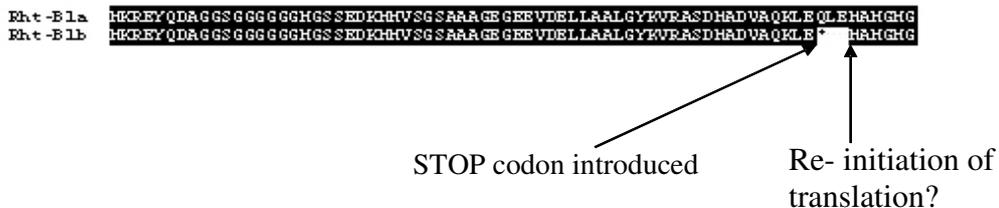


Figure 1.2 Partial amino acid sequence of the wheat protein encoded by the Rht-B1a and Rht-B1b loci with amino acid internal deletions of the allele causing the semi-dwarf phenotype.

Dwarfing genes are grouped on their response to applied GA. Mutants of the biosynthetic pathway, such as rice *sd-1*, are GA sensitive and the phenotype of the wild-type can be restored by exogenous application of GA. Dwarf mutants of the GA response, such as wheat *Rht-B1b* and *Rht-D1b*, the maize *dwarf 8 (D8)* or its ortholog in sorghum *dwarf3 (dw3)* and the *Arabidopsis* GAI, are insensitive to applied GA. In recent years more mutants related to either *sd-1*, *Rht* or other genes in GA metabolism have been identified causing dwarfism in wheat and rice (Milach *et al.*, 2002; Xu *et al.*, 1995; Carrera *et al.*, 2000; Hedden *et al.*, 1998).

In gibberellin response mutants, three main classes of mutations have been identified affecting plant height. These are (a) gibberellin-insensitive dwarfs, (b) gibberellin-deficient mutants in which the plants can be reversed closer to normal by co-expression of a second “suppressor” mutation, and (c) mutants with a constitutive gibberellin response also called “slender” mutants (Taiz and Zeiger, 2006). Examples for gibberellin-insensitive dwarfs are the wheat “green revolution” mutants with a mutation in the *Rht-1* and *Rht-2* genes and their orthologs in maize, *Dwarf8 (D8)*, and in *Arabidopsis*, *Gibberellic acid Insensitive – GAI*, were also found to confer a semi-dwarf phenotype. The *Rht* locus encodes a repressor protein (GAI) in *Arabidopsis* inhibiting stem elongation in the absence of GA. The *Arabidopsis* dwarf mutant (*gai*) protein has a 17 amino acids deletion rendering it insensitive to foliar application of GA (Fu *et al.*, 2001 and Peng *et al.*, 1999).

A mutation in the rice *sd-1 (GA20ox-2)* coding region (280 bp deletion) is a loss of function mutation causing a semi-dwarf phenotype when the mutated gene was expressed in other crops such as *Arabidopsis* and potato (Spielmeyer *et al.*, 2002). Rice plants with the mutation also had a greater harvest index allowing for increased use of nitrogen fertilizers. However,

the presence of multiple *sd-1* alleles prevented severe dwarfing due to a partial inhibition of GA production (Yamaguchi, 2008). In *Arabidopsis*, which carries three GA20 oxidases, such functional redundancy after mutation, and with GA still produced in other plant parts, caused a semi-dwarf phenotype (Spielmeyer *et al.* 2002).

1.3.2 Manipulation of plant height using GA genes

The key role shown above that GA metabolic genes play in plant architecture has made them prime targets for genetic manipulation. Characterization of these genes has paved the ground for geneticists and physiologists to target specific metabolic pathways in the production of higher yielding and hardier plants. However, from the agronomic point of view, not all the genes involved are of interest (Yamaguchi, 2008; Hedden and Phillips, 2000). Changing or manipulating the endogenous bioactive GA amount allowed the design of crops with a better morphological architecture. This approach offers an alternative strategy to introduce beneficial traits, such as dwarfism, into cereal varieties to improve grain yield.

Modifying the endogenous amount of bioactive GA might occur either through genes contributing to the production of the bioactive GA or through genes diverting GA forms to inactive molecules. This includes catabolic inactivation of bioactive GA forms or some of its precursors (Hedden and Phillips, 2000). Studies by Sun and Kamiya (1994) and Fleet *et al.* (2003) have shown that over-expression of the genes encoding enzymes that catalyse the early stages of GA biosynthesis, e.g. *ent*-copalyl pyrophosphate synthase (*AtCPS*) and *ent*-kaurene synthase (*AtKS*) in *Arabidopsis*, do not significantly increase amounts of bioactive GA with no effect on plant growth and development. Over-expression of genes downstream pathway, such as the GA 20-oxidases that are multifunctional and highly regulatory enzymes

(Figure 1.4), increased stem elongation, early flowering and decreased seed dormancy indicating that their activity limits GA biosynthesis (Lang, 1998). Suppression of the different GA 20-oxidase homologous genes in *Arabidopsis* through RNAi expression produced changes in the different parts of the plant showing their tissue specific role (Coles *et al.*, 1999). Over-expression of its own GA 20-oxidase in *Arabidopsis* resulted in the elongation of seedling hypocotyls, increased shoot growth, induced early flowering, and increased GA₄ level (Huang *et al.* 1998; Coles *et al.* 1999). However, over-expression of the same gene from citrus or *Arabidopsis* caused an increased amount of bioactive GA and elongated phenotypes in hybrid aspen and tobacco plants (Eriksson *et al.*, 2000; Vidal *et al.*, 2001; Biemelt *et al.*, 2004). In potato, Carrera *et al.* (2000) showed that antisense mRNA expression of a *GA20ox* gene reduced stem elongation and increased both tuberization and tuber yield. Studies by Israelsson *et al.*, (2004) and Phillips (2004) further indicated no difference in the morphology of transgenic plants following GA 3-oxidase over-expression in hybrid aspen and *Arabidopsis*. In rice, antisense copies of *GA3ox2 (D18)* reduced the final GA amount and caused semi-dwarf phenotypes in some of the transformants (Itoh *et al.*, 2002). This phenotype, however, was not stably transferred to the progeny possibly due to gene silencing.

Lowering of the endogenous GA amount is also possible through increasing the expression of GA 2-oxidase, a positive feed forward regulation enzyme, which catabolises bioactive GAs and some precursors. After its first isolation using cDNA from runner bean (*Phaseolus coccineus*) by a functional screening method (Thomas *et al.*, 1999), an ectopic expression of *OsGA2ox1* gene in rice resulted in reduced stem growth with small, dark green leaves with reproduction organs severely defective (Sakamoto *et al.*, 2001). However, expression of the same gene under the control of the shoot-specific *OsGA3ox2* promoter induced only a semi-dwarf phenotype with normal flower and grain development (Sakamoto *et al.*, 2003).

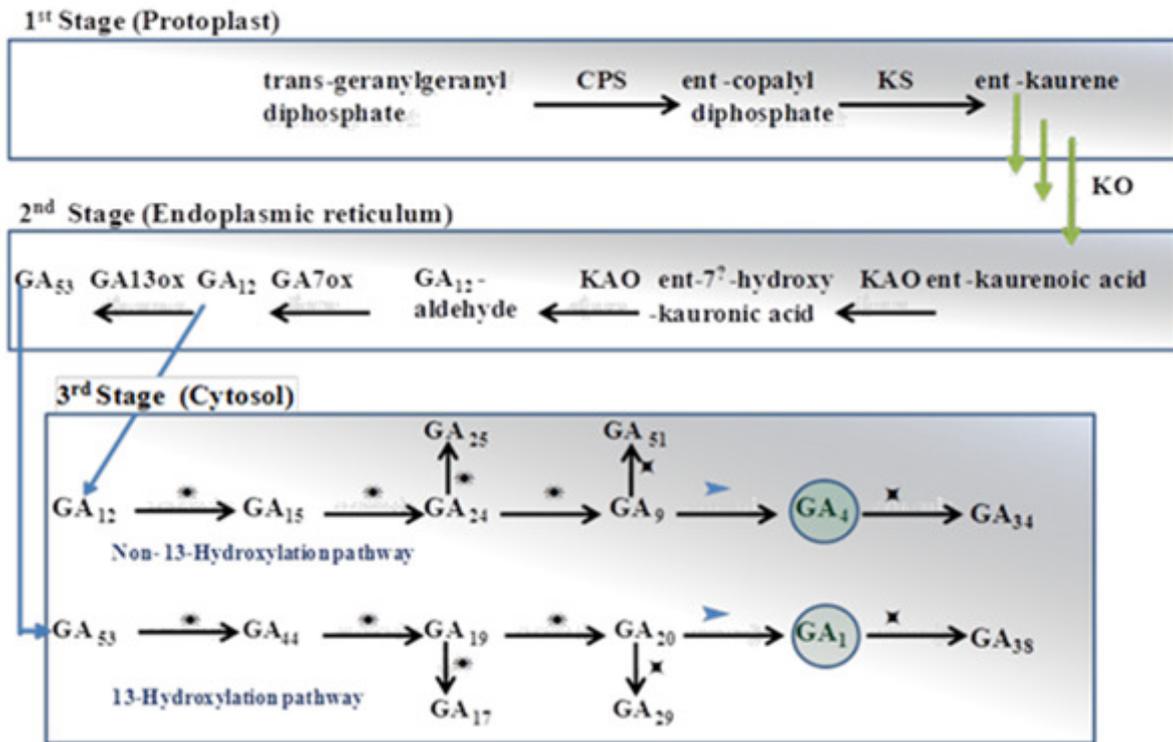


Figure 1.3 Simplified pathway of the gibberellin (GA) biosynthesis and deactivation in plants. Most targeted genes in inducing dwarfism are the 2-ODD family multifunctional genes catalyzing several steps at the intermediate (*) and late (✕) steps of the biosynthesis pathway catalyzed respectively by GA20ox and GA2ox. In addition to inactivating the bioactive GAs (GA₁ and GA₄), the GA 2-oxidase also deactivates intermediates GA₉ and GA₂₉. (Adapted from Oikawa *et al.*, 2004)

Severe dwarf phenotypes were obtained in *Arabidopsis*, tobacco and poplar through over-expression of the rice GA 2-oxidase gene (Schomburg *et al.*, 2003; Biemelt *et al.*, 2004; Busov *et al.*, 2003). Plants with a dwarf phenotype were also produced by over-expression of a runner bean GA 2-oxidase in *Arabidopsis* and wheat and Hedden and Phillips (2000) suggested the superiority of this approach for the breeding of dwarf plants.

1.4 **Brassinosteroid genes and lodging**

Mutated genes of the brassinosteroid metabolism also causes a dwarf phenotype. The *brachytic2* (*br2*) mutant in maize and its ortholog in sorghum, *dwarf3* (*dw3*), induce short internodes (Multani *et al.*, 2003). In barley, *uzu* dwarfism caused by the missense mutation in the *HvBR11* gene is a mutation in the brassinosteroid receptor protein resulting in a semi-dwarf phenotype (Chono *et al.*, 2003). Several other BR deficient and BR-insensitive mutants have been identified with phenotypic changes including dwarfism, small dark-green leaves, a compact rosette structure, delayed flowering and senescence and reduced fertility (Sasse, 2002). The practical use of the BR has been limited due to BR deficiency leading to severe and defected dwarfism and reduced fertility (Divi and Krishna, 2009). However, controlled changes i.e. slight decrease in BR levels or in BR signaling was found causing significant increase in yield as a result of change in plant architecture (Divi and Krishna, 2009).

In monocots, only BR-insensitive mutants have been identified (Yamamuro *et al.*, 2000.). A rice mutation in the C-22 hydroxylase, a BR enzyme involved in leaf inclination, resulted in a semi-dwarf phenotype that increased above ground biomass by 40% (Sakamoto, 2006). In tomato, a BR-responsive *dwarf* (*d*) mutant was found caused by inactivation of a cytochrome P450 enzyme (CYP85A1) (Bishop *et al.*, 1996). In *Arabidopsis*, overexpression of *DWF4*, a

gene that encodes a cytochrome P450 monooxygenase (CYP90B1) (Choe *et al.*, 1998) resulted in a dramatic promotion of vegetative growth and enhanced seed yields (Fujioka and Yokota, 2003). In barley, an ortholog of BRI1, a BR-insensitive mutant due to single nucleotide change in the BR-receptor gene produced a semi-dwarf phenotype with increase in yield and lodging resistance. Generally, the current knowledge on BR regulation of growth and development through altered BR activity is growing rapidly through characterization of a wide variety of BR-deficient and BR-insensitive mutants (Fujioka and Yokota, 2003).

1.5 Induced mutations

Inducing mutation in crops has been long exercised to create variability in germplasm for species and traits where there is little known variation for a trait of interest. In *E. tef* the genetic diversity for lodging resistant traits has not been found to exploit through genetic introgression into modern cultivars. In other cereal crops, however, inducing mutation to improve genetic diversity have brought about renewed interest because it mimics natural variation with relatively high frequency in the selected germplasm with desirable genetic backgrounds (Maluszynski and Szarejko, 2003). The technique provides novel genes or alleles (Table 1.1) of known phenotypes (Maluszynski and Szarejko, 2003) in better adapted or a more desirable background (Konzak, *et al.*, 1984). Through induced mutations, many genes involved in metabolic pathways responsible for plant development and growth, response to growth regulators and various biotic and abiotic stresses have been identified (Barkley and Wang, 2008; Maluszynski and Szarejko, 2003). The most obvious and attractive feature in inducing mutants is ease of inducing the genetic variation with relatively high frequency, and very often the mutations mimic natural variations. The genetic changes are made in advanced genotypes or in genotypes adapted to local environmental conditions or

having desirable background traits. Among economic traits largely targeted for improvement using this technique include semi-dwarfness for conferring lodging resistance in cereal crops (Barkley and Wang, 2008; Hu, 1973; Ullrich and Aydin, 1985; Maluszynski *et al.* 2003).

Semi-dwarfness is one of the most desirable traits targeted by induced mutations due to lack of diversity in desirable phenotypic traits in the genetic pool of many important crops. In rice, several semi-dwarf mutants, including stiff and lodging resistant, have been selected from mutated populations and led to the release of important new varieties (Maluszynski and Szarejko, 2003). Developing and selecting useful mutations involve random mutations for qualitative traits coupled with large screens of the mutated plants. This requires sufficient time (1- 2 years) and development of a high quality population. Such technical challenges in mutation technologies render the method less attractive to many scientists (Barkley and Wang, 2008; Baenzinger, 1988). In barley, a new semi-dwarf lodging resistance mutant variety has been selected with an average mature height reduced to 87 cm from a tall, 120-130 cm, phenotype. This increased yield by about 15% and with high input about 25% (Maluszynski and Sigurbjörnsson, 1988; Rutger, 1981). Inducing dwarfism or reduced plant height without losing the potential yield has been reported for wheat and other cereals (Table 1.1) (Barabäs and Kertész, 1988; Narahari, 1988).

Spontaneous mutation occur with an extremely low frequency, often unnoticed being difficult to detect in species like the tetraploid *E. tef*. Thus genetic manipulation such as inducing mutation in target genes using various mutagens can provide rapid generation and enhancement of genetic variability. Inducing short stature mutants without changing the background character of important traits will be extremely beneficial for developing lodging resistance in *E. tef*.

Table 1.1 Semi-dwarf sources in *Rht* wheat induced by chemical or physical mutagens (Maluszynski *et al.* 2001).

Gene	Source	Parent variety	Mutagen	Breeding value
Rht4	Burt	M937	Gamma rays	
Rht5	Marfed M1	Marfed	EMS	
Rht7	Bersee Mut	Bersee	EMS	
Rht11	Krasnodarskii karlik	Bezostaja	MNH	***
Rht12	Karcag 522M7K	Karcag 522	Gamma rays	
Rht13	Magnif 41M1	Magnif 41	MNH	
Rht14	Cp B144	Cappelli	thN	***
Rht15	Durox	K6800707	EMS	*
Rht16	Edmore M1	Edmore	EMS	*
Rht17	Chris M1	Chris	DES	
Rht18	Icaro	Anhinga	fN	*
Rht19	Vic M1	Vic	EMS	*
Rht20	Burt M860	Burt	Gamma rays	*

*** high breeding value.

EMS= ethylmethane sulfonate; DES = ; MNH =
Source: Maluszynski and Szarejko, 2003

1.6 Plant growth regulators for plant height control

Plant height, particularly culm length, is considered to be among the major factors associated with lodging sensitivity (Pinthus, 1973; Crook and Ennos, 1994; Berry *et al.*, 2000). Control of plant growth, such as plant or culm height, can be achieved chemically by using plant growth regulators (PGRs). Many chemical growth promoters or retardants have been used to treat crops and plants for controlling growth and development of vegetative or reproductive parts. PGRs that inhibit gibberellins (GA) biosynthesis are used in high input cereal management to shorten straw and thereby increasing lodging resistance. They 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). Among the GA inhibitors that are used to control plant growth are the onium-type compounds, such as chlormequate chloride (2-chloroethyl-N,N,N-trimethyl-ammonium chloride, CCC) and mepiquat-Cl, interfering with *ent*-kaurene synthesis at the early stages of gibberellin biosynthesis (Rademacher, 2000) (Figure 1.1). Inhibition of the cyclization of geranylgeranyl diphosphate synthase (GGPP) into copalyl diphosphate synthase (CPP) due to CCC binding to the enzyme CPP-synthase reduces the availability of bioactive GA (Hedden and Philips, 2000; Graebe *et al.*, 1992; Rademacher, 2000).

A further group are nitrogen containing heterocycles such as *triazoles* and *imidazoles*. This includes paclobutrazol (PBZ) and the closely related uniconazole-P. Both compounds interfere in the oxidation of *ent*-kaurene to *ent*-kaurenoic acid (Rademacher, 2000). Inhibition of oxidation of mono-oxygenases occurs by sharing lone pair electrons to displace oxygen from the enzyme binding site at the proto-heme iron, this renders the oxygenase non-functional (Rademacher, 2000) (Figure 1.1). Commercially available PBZ in the (2*S*,3*S*)-

enantiomer is structurally similar to *ent*-kaurene. More recently developed GA inhibitors include cimectacarps (trinexapac-ethyl), interfering with the late stages of GA metabolic reactions mainly by inhibiting 3 β -hydroxylation of GA₂₀ to produce the bioactive GA₁ (Rajala, 2003). In general, the stem growth inhibition due to PGRs can be variable, depending on species and genotypes (Rajala, 2003), and is further based on GA inhibitor mediated stem shortening by interfering with synthesis of an intermediate precursors, *ent*-kaurene or *ent*-kauronic acid, or by inhibiting 3 β - hydroxylation of GA₂₀ to bioactive GA₁ (Graebe *et al.*, 1992; Rajala, 2003; Hedden *et al.*, 2010).

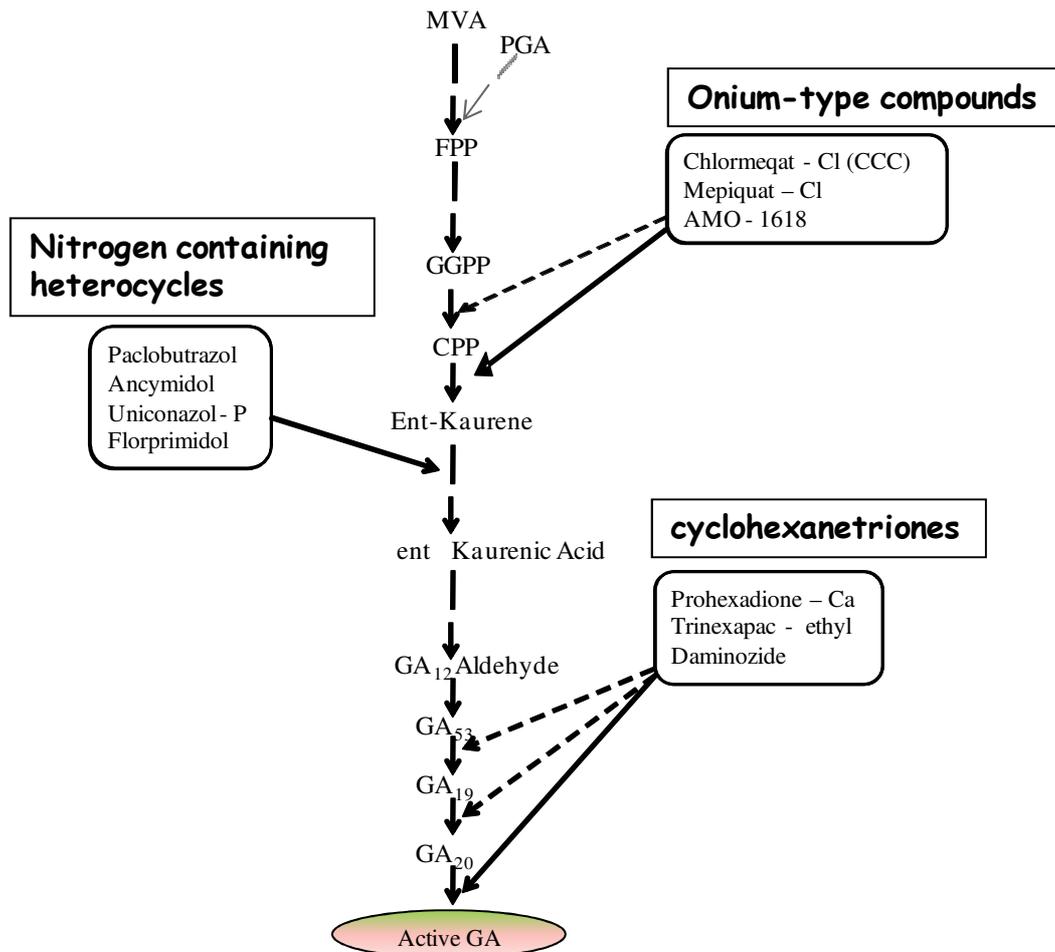


Figure 1.4 Simplified scheme of GA biosynthesis steps and points of inhibition by plant growth regulators. Broken line represents minor inhibitor activities (Rademacher, 2000).

Reduction in plant height following application of PGRs is associated with reduced endogenous bioactive GA amounts and reduced elongation of internodes particularly of the uppermost internodes and peduncle (Sanvicente *et al.*, 1999; Rajala, 2003). CCC inhibits stem elongation in wheat by reducing up to 40% plant height (Humbries *et al.*, 1965). In oilseed rape, foliar treatment with a combination of CCC, ethephon and imazaquin reduced main stem length by 7% in the field and 16% under greenhouse conditions. The uppermost three internodes contributed significantly to the reduction (Sanvicente *et al.*, 1999). In barley,

responses to CCC were genotype-dependent (Rajala, 2003), but when wheat or barley plants were treated with CCC, the PGR had no effect when plants already contained dwarfing genes such as *Rht1*, *Rht2*, or *Dw6* (Abbo *et al.*, 1987; Peltonen-Sainio and Rajala, 2001). PBZ application reduced stem length in some rice cultivars by 90%, lodging from 60% (in controls) to 0% and increased yields up to 15% when compared to controls (French *et al.*, 1990). PBZ and the closely related uniconazole-P are highly active PGRs with practical uses in rice, fruit trees and ornamentals (Rademacher, 2000) and about 84% of the winter wheat in UK is treated with PGRs (Berry *et al.*, 2004).

PGR application (CCC treatment) has been found to increase lodging resistance due to an increase in stem diameter (Tolbert, 1960). However, other research groups showed no change in the content of structural compounds (cellulose, lignin and hemicelluloses) in the plant stem following CCC and ethephon treatment (Clark and Fedak, 1977; Knapp *et al.*, 1987). In barley, no clear relationship was found between stem diameter and cell wall thickness of the two basal internodes with lodging susceptibility (Stanca *et al.*, 1979). Shortening of stem after PGR treatment may also not necessarily result in reduced lodging as reported for wheat and barley (Knapp *et al.*, 1987; Ma and Smith, 1992).

Information about the use of PGRs in *E. tef* is very limited but increased yield following CCC application at 0.7 - 2.0 l a.i. ha⁻¹ has been reported but lodging was not prevented (Alkamper, 1970). This early *E. tef* result has been further supported by Berry *et al* (1998) applying CCC to a lodging-prone crop and reduced lodging area only from 88 to 83% at harvest.

1.7 Lodging in *E. tef*

1.7.1 *E. tef* growth

Tef, *Eragrostis tef* (Zucc.) Trotter, is a small-seeded full grain cereal with high economic importance in Ethiopia. It is the most resilient crop with low risk of failure (Tefera and Ketema, 2001) grown under very diverse environments and exhibits high diversity in most pheno-morphic and agronomic traits (Assefa, 2003). In Ethiopia, *E. tef* is grown on over 2.56 million ha¹, accounting for about 28% of the total acreage and 19% of the gross grain production of the major cereals (CSA, 2008). The lives of estimated over 50 million people depend directly on *E. tef* as a staple food. *E. tef* grows on water-logged vertisol in the highlands as well as water-stressed areas in the semi-arid regions (Takele *et al.*, 2000). Suitable growing rain-fed areas are reported to be those with a growing period of 100 - 150 days, rainfall of 375 - 700 mm and a mean temperature of 12 - 22°C (Takele *et al.*, 2000). However, Kebede *et al.* (1989) reported that higher dry matter accumulation occurs at 35°C than at 25°C with the highest leaf carbon exchange rate, 31.8 $\mu\text{-mol m}^{-2} \text{s}^{-1}$, occurs at this temperature.

Grain yield varies from 1-2.5 t ha⁻¹ with a national average yield of about 1.0 t ha⁻¹ and grain yield potential which might be elevated to 4.5 t ha⁻¹ (Tefera and Belay, 2008; Teklu and Tefera, 2005). Yield ranges between 2.5- 4.5 t ha⁻¹ have been reported for research plots using improved varieties and with support of a net to prevent lodging (Tefera *et al.*, 2001; Mamo and Parsons, 1987; Delden *et al.*, 2010). Early local varieties maturing in less than 85 days, such as Gea-Lamie, Dabi, Shewa-Gimira, Beten and Bunign, are widely used under short growing conditions experiencing low moisture stress in the mid and low altitude or low

temperature at high altitudes. Under a suitable growing environment, local cultivars, such as Alba, Ada and Enatit, are used. Modern varieties, such as DZ-01-354, DZ-01-196, DZ-01-787, are widely grown by farmers in areas with optimal rainfall and DZ-Cr-37 is grown in low-moisture stress areas. These varieties give mean grain yields ranging from 1.4 to 2.7 t ha⁻¹ (Assefa, 2010; Ketema, 1997; CSA 2008).

1.7.2 Pheno-morphic features related to lodging

Lodging is a key agronomic problem in *E. tef* production (Yu *et al.*, 2007) and up to 23% yield loss is accountable to lodging under natural conditions (Ketema, 1983) i.e. with minimal or no fertilizer condition. Even with good crop management practices, lodging is a major limitation to sustainable improvement of the crop. *E. tef* generally has a tall culm height up to 155 cm and a fine or slender stem with first and second basal culm internode diameter range from 1.2-4.5 mm (Aseffa *et al.*, 2010; Teklu and Teferea, 2005; Ketema, 1983). Thus *E. tef* is characterized by a low root-collar diameter to plant-height ratio. Nearly all improved varieties have a tall phenotype with culm height reaching up to 150 cm and with a basal internode diameter of <4.5 mm (Figure 1.5). The root system is fibrous and shallow emerging from nodes above the base, and growing 4 - 8 cm deep under field conditions. The panicle forms about a third of the culm length (Ketema, 1997; Kebede *et al.*, 1989). Most of the above characteristics appear to be typical making the crop very susceptible to lodging (Figure 1.5) due to weak stem-base having insufficient strength to hold the shoot up against leverage.

A



B



Figure 1.5 *E. tef* plant stand in the field at (A) grain filling and (B) at maturity when almost all plants lodged (Source: Dr. Likyelesh Gugssa (Holetta Agricultural Research Center)).

Most studies showed stem lodging due to bending at the basal internodes to be the major problem in *E. tef* (Ketema, 1983; Asefa *et al.*, 2000)). In a modelling work for the lodging character in *E. tef* Mark (1985) took into account node diameter, 1st and 2nd internode length, biomass and wind among external forces acting on the plant. According to him the lodging score (S) is computed as:

$$S = \frac{b_0 + b_1 h (W + Q)}{D^3 (1-t^4)}, \text{ where:}$$

S = lodging score; **b₀**, **b₁** = empirical constant; **W** = tiller weight, **Q** = drag force due to wind, **t** = node diameter ratio (inner: outer); **D** = mean internode length (1st & 2nd). Because of a high correlation of panicle length with yield and hence tiller weight (Mengesha, 1965), panicle length has been substituted for (W + Q) and mean diameter of the 1st and 2nd for internode length. However, this model could predict only about 33% of the variance recorded for lodging implicating possible involvement of other factors not accounted for. Further development of models to predict and improve the lodging character in *E. tef* has not been made except in a recent study to examine applicability of other crops' models developed for wheat and barley (Delden, 2010).

1.7.3 *E. tef* breeding for lodging resistance

E. tef breeding has mainly resulted in tall phenotypes with low root collar diameter to plant height ratio (Ketema, 1983) and most varieties so far developed for high yield have this tall phenotype (Teklu and Teferea, 2005). No genotype has been found so far to be lodging resistant and there is no clear agreement on the important trait to look for. Berhe (1981) regarded short, stiff-strawed genotypes as important, others suggest short plants (possibly

straw + panicle) (Ketema, 1983; Mengesha *et al.* 1965) and they reported a high correlation for lodging with stem diameter, plant height, panicle length and yield. Hundera *et al.* (1999) reported days to heading and maturity to be negatively associated with lodging while plant height, culm length, panicle length, culm diameter, panicle weight, and shoot biomass were highly significant and positively associated with lodging resistance. Asefa *et al.*, (2000) recommended stem morphology related characters, such as total height, number of nodes, thickness and length of basal internodes, to be important. Teferra *et al.* (2003) reported that high yielding lines tend to lodge more severely because of failure to bear the heavy panicles, indicating that lodging also imposes limitation on genetic improvement in *E. tef* for further yield increase. Recent studies in *E. tef* have shown strong correlations between lodging, panicle type, culm thickness, and grain yield (Yu *et al.*, 2007). Lodging index showed positive and highly significant correlations with primary shoot weight, 100 seed weight, grain yield, shoot biomass and negative correlations with peduncle length thus, high yielding lines tended to lodge. The positive and strong relationship of lodging with plant height and plant height with yield and other important yield component traits indicates lodging resistance improvement will remain challenging in *E. tef* until it is possible to uncouple plant height and yield traits. Overall, lack of knowledge of exact traits to look for is still the most critical drawback in modern *E. tef* cultivation.

Van Delden *et al.*, (2010) also reported that *E. tef* has the lowest value for plant base diameter, the diameter of tillers at the soil surface, and the average root plate diameter compared to other cereals like wheat and rice and emphasized the significance of root failure as yet another serious factor in *E. tef* lodging. However, it is not yet clear if root lodging could well be associated with *E. tef* root morphological attributes such as root strength and rigidity, root number and length, or stem characters like thicker stem base. Moreover, how

these factors interact with different soil characteristics to cause the lodging problem needs to be investigated.

1.8 Working hypothesis and aim of study

In this PhD study the problem of reducing plant height in *E. tef* was addressed to improve lodging resistance in the crop. Since the GA metabolism plays a significant role in plant height control, it was hypothesized that regulation of the GA amount in *E. tef* will change pheno-morphic and also agronomic characteristics that will affect lodging and also decoupling plant height from yield. This study had therefore the aim to reduce plant height by either chemical PGR treatment or manipulation of gene expression to reduce plant height and also to study in more detail the expression of height regulating GA genes in *E. tef*. The objectives of the study were (i) to study the *in vivo* response of two *E. tef* genotypes (short variety: Gea Lammie and long variety: DZ-01-196) to treatment with PGRs to confirm a role of GA in *E. tef* plant height control, (ii) to optimize *E. tef* plant transformation and regeneration for the production of transformed *E. tef* plants with reduced GA content (iii) to characterize transformed *E. tef* plants over-expressing GA2-oxidase (*PcGA2ox1*) under the control of a CaMv3x35S promoter to decrease bioactive GA amounts (iv) to identify and characterize the genes involved in plant height control (rice *sd-1* and wheat *Rht* orthologous genes) in *E. tef* and (v) to characterize morphologically and physiologically existing semi-dwarf *E. tef* mutants derived from a TILLING process.