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Physiological Evidence of Rht1 and Rht2 Genes Expression in few Indian Wheat Cultivars and studying their behaviour in response to exogenous GA_3 application

Sood Swati[1], Singh Rupinderpal[2], Chatli S Anshu[3], Mehta Sonam[4] , Kaur Amandeep[5]

[1]Department of Biotechnology, Guru Nanak Girls College, Ludhiana-141002, India.
[2]Department of Plant Breeding & Genetics, PAU, Ludhiana-141004, India.
[3]Department of Microbiology,Guru Nanak Girls College, Ludhiana-141002, India.
[4] Department of Biotechnology, Guru Nanak Girls College, Ludhiana-141002, India.
[5] Department of Biotechnology, Guru Nanak Girls College, Ludhiana-141002, India.

ABSTRACT

The most common dwarfing genes in wheat (Triticum aestivum), Rht-B1b and Rht-D1b, classified as gibberellin-insensitive (GAI) dwarfing genes due to their reduced response to exogenous GA, have been verified as encoding negative regulators of gibberellin signalling. The introduction of the reduced height Rht-B1b and Rht-D1b semidwarfing genes led to impressive increases in wheat (Triticum aestivum) yields during the Green Revolution. The reduction in stem elongation in varieties containing these alleles is caused by a limited response to the phytohormone gibberellin (GA), resulting in improved resistance to stem lodging and yield benefits through an increase in grain number. The phytohormone gibberellin (GA) regulates many plant growth and developmental processes. Rht-B1 and Rht-D1 encode DELLA proteins, which act to repress GA-responsive growth, and their mutant alleles Rht-B1b and Rht-D1b are thought to confer dwarfism by producing more active forms of these growth repressors. The Gibberellin insensitivity of Rht-B1b and Rht-D1b was confirmed by studying the response of Rht-B1b and Rht-D1b to exogenous GA_3 treatment, on coleoptile length using few Indian wheat cultivars. The application of exogenous GA_3 had no significant effect on coleoptile length of genotypes expressing Rht-B1b and Rht-D1b genes whereas the coleoptile length of genotypes lacking these genes increased significantly upon exogenous GA_3 treatment. It indicates that these newly characterized mutations in Rht-B1 and Rht-D1 confer "GA-insensitive" dwarfism by producing DELLA proteins that do not bind the GA receptor GA INSENSITIVE DWARF1, potentially compromising their targeted degradation.

Keywords : wheat (Triticum aestivum), dwarfing genes, gibberellins, lodging, DELLA proteins, coleoptile length

INTRODUCTION

Among several constraints towards realizing the potential yield in wheat, the lodging is one of the major threats to wheat production worldwide including India. Lodging in cereal crops is influenced by morphological (structural) plant traits as well as environmental conditions. Lodging in cereals is often a result of the combined effects of inadequate standing power of the crop and adverse weather

conditions, such as rain, wind, and/or hail. Lodging is also variety (cultivar) dependent. For example, a tall, weak-stemmed wheat cultivar has a greater tendency to lodge than a semi- dwarf cultivar with stiffer straw. Under conditions of high moisture and nitrogen fertility, semi-dwarf varieties are less prone to lodging than standard ones. Furthermore, short thick-strawed cultivars resist lodging better than tall cultivars.

In wheat, there are twenty-one major genes which have been reported to affect plant height and assigned designations from *Rht1* to *Rht21*(McIntosh et al., 2008).The introduction of semidwarfing genes, Rht-1 (Rht-B1b) and Rht-2 (Rht-D1b), both derived from 'Norin 10', into wheat (*Triticumaestivum*) permitted the breeding of higher yielding varieties during the Green Revolution (Hedden, 2003). 'Green revolution' genes Rht-B1b and Rht-D1b were mappedon the short arms of chromosomes 4B and 4D, respectively. The higher yields were due to shorter, sturdier straw which increased lodging resistance and harvest index (Gale and Youssefian, 1985). The decrease in stem stature resulted in an increase and efficient utilisation of available assimilates and also directed them towards developing ears which increased grain numbers per ear (Youssefian et al., 1992).

In many plant species, growth is mediated by GA phytohormones. This class of plant hormones has a well-characterized role in controlling stem elongation (Ross, 1994).In wheat, dwarfing genes are classified into two main categories, GA-responsive and GA-insensitive on the basis of their response to exogenous GA application. This difference can be analysed at the seedling stage for example on the basis of response of coleoptile length to exogenous GAs application. GA-responsive genes show enhanced growth response upon exogenous GA application and GA-insensitive genes (e.g. Rht-B1b and Rht-D1b) do not show any significant growth response upon exogenous GA application.

Reduced plant height conferred through the introduction of semidwarfing Rht genes Rht-B1b and Rht-D1b, is due to decreased responsiveness to GA phytohormones (Gale and Marshall, 1973; Pinthus et al., 1989).The Rht-B1b and Rht-D1b genes encode for DELLA proteins, transcriptional regulators which act to repress GA signalling. The DELLA protein belongs to a plant specific family of transcription factors known as GRAS. The mutant Rht allelesRht-B1b and Rht-D1b have single nucleotide substitution which led to non-sense mutation i.e. introduction of premature stop codons in the N-terminal coding region. This led to the termination of translation within the DELLA region, resulting in production of N-terminally truncated proteins, which are more active growth repressors and confer dwarfism by repressing GA signaling (Peng et al. 1999).

In many cases, environmental factors lead to the increased production of bioactive GAs which cause the targeted degradation of the DELLA proteins (Yamagichi,2008; Sun, 2010).This targeted degradation of DELLAs is initiated by the binding of GA to GA receptor, GA INSENSITIVE DWARF1 (GID1) within its pocket. This binding leads to the binding of DELLAs through their conserved DELLA/TVHYNP motifs. GID1/DELLA binding enables the recognition by the GID2/SLEEPY1 F-BOX component of SCF Ub E3 ligase, leading to polyubiquitination of DELLAs and their subsequent degradation through 26S proteasome(Fu et al., 2002; Sasaki et al., 2003; Dill et al., 2004; Griffiths et al., 2006; Feng et al., 2008; Hirano et al., 2010). The mutations in DELLA region resulted in reduced GA sensitivity and hence reduced plant height. The mutations are produced in the DELLA/TVHYNP motif region which restricts the binding of DELLA to GID1 receptor, protecting DELLAs from degradation and hence confers dwarfism through GA signaling repression.

MATERIALS AND METHODS

General description

The experiments were carried out in the department of biotechnology, Guru Nanak Girls College, Ludhiana. The Rht-B1b and Rht-D1b gene expression of four wheat genotypes provided by Punjab Agricultural University, Ludhiana, was studied at the physiological level. Also, the growth response of these genotypes to exogenous GA_3 application was studied.

Plant Material

Seed stocks of eight wheat genotypes, HD3086, HD2967, WH1105, C323, C591, C518, C306, PBW 550 were obtained from PAU, Ludhiana, Punjab.

Experiment 1: Coleoptile length and root length

Coleoptile length and root length was measured using 'Cigar' method. In this method, seeds were placed on the line drawn in the middle of the germination paper wetted with water. The germination paper is folded in the form of rolled cigar, with a seed in each fold. The rolled cigars were placed in vertical position at 22°C and were irrigated with water properly at intervals. Coleoptile length and root length were measured from the seed to the tip of the coleoptile and root respectively, with a ruler. Length was measured on 10^{th} and 15^{th} day after germination.

Experiment 2: Exogenous Gibberellic Acid (GA_3) Treatment

The above mentioned cigar method was followed in this experiment also. The Experiment 1 was repeated but with a difference that rolled cigars placed in vertical position at 22°C were irrigated with GA_3 solution (100 mM or 35 mg/L) instead of water. Coleoptile length and root length were measured on 10^{th} and 15^{th} day after germination.

Experiment 3: Plant height character

At maturity, five random plants for each genotype were measured to get the mean plant height as the distance from the soil surface to the top of the ear (awns excluded).

RESULTS AND DISCUSSION

On the basis of coleoptile length and root length measurements (Table 1), it is proved that the genotypes HD 3086, WH 1105, HD 2967 and PBW 550 have the genes for reduced plant height Rht-B1b and Rht-D1b and the genotypes C323, C591, C518 and C306 lack the genes for reduced plant height. The genotypes HD 3086, WH 1105, HD 2967 and PBW 550 have shorter coleoptiles as compared to the genotypes C323, C591, C518 and C306, which have long coleoptiles. Also, these genes have proved to be GA-insensitive as there was no significant effect on the coleoptile length of the genotypes containing these genes upon exogenous GA_3 application, whereas the genotypes lacking these genes showed significant increase in their coleoptile lengths upon exogenous GA_3 application (Table 2 & 3). Also, these Rht genes containing genotypes have reduced plant height character as compared to the other genotypes (Table 4). So, due to their reduced plant height character, these genotypes have found to be lodging resistant.

TABLES

Coleoptile length and root length

Table 1: coleoptile length and root length of eight wheat genotypes irrigated with water by using Cigar method

S.No.	Genotypes	10^{th} day		15^{th} day	
		Coleoptile Lenght (Cm)	Root Lenght (Cm)	Coleoptile Lenght (Cm)	Root Lenght (Cm)
1.	HD 3086	8.2±1.03	13±1.10	10.4±1.46	12.4±2.06
2.	C 323	12.2±1.44	10.4±1.36	19.4±3.20	15.34±0.38
3.	WH 1105	8.9±2.22	9±0.84	13.7±2.40	13±3.16
4.	C 591	15.8±2.14	8.9±0.58	26.8±3.06	14.6±0.49
5.	HD 2967	13±1.79	12.6±2.87	19.4±2.42	15.2±2.64
6.	C 518	17.8±5.74	9.6±1.36	25.6±5.64	12.4±1.62
7.	C 306	24.84±5.12	11.8±1.33	28.2±3.16	14±0.89
8.	PBW 550	19.2±5.81	14.2±1.94	20.2±1.33	15.4±3.14

All data are means ± SD of each group. ±SD (standard deviation)

Table 2: coleoptile length and root length of eight wheat genotypes irrigated with GA_3 solution by using Cigar method

S.No.	Genotypes	10^{th} day		15^{th} day	
		Coleoptile Lenght (Cm)	Root Lenght (Cm)	Coleoptile Lenght (Cm)	Root Lenght (Cm)
1.	HD 3086	6.98±0.57	12.4±1.36	9.9±0.51	12.8±0.48
2.	C 323	20.94±0.82	23.6±5.78	23.9±2.35	26.4±5.5
3.	WH 1105	9.96±0.84	15.2±2.04	12.6±2.33	19.14±2.52
4.	C 591	25.18±0.57	25.8±5.76	28.2±1.08	22.9±2.5
5.	HD 2967	14.3±1.25	11.1±0.82	18.3±1.70	14.4±1.02
6.	C 518	15±0.82	8.4±1.61	15.8±1.01	14.7±0.50
7.	C 306	14.4±1.18	7.7±1.16	28.2±1.93	15±0.82
8.	PBW 550	14.7±0.52	12±0.82	30.7±0.52	15±0.74

All data are means ± SD of each group. ±SD (standard deviation)

Table 3: Comparison of coleoptile length and root length of eight wheat genotypes irrigated with water and GA_3 solution by using Cigar method

S.No.	Genotypes	Coleoptile Lenght (Cm)		Root Lenght (Cm)	
		Water	GA_3 treated	Water	GA_3 treated
1.	HD 3086	10.4±1.46	9.9±0.51	12.4±2.06	12.8±0.48
2.	C 323	19.4±3.20	23.9±2.35	15.34±0.38	26.4±5.5
3.	WH 1105	13.7±2.40	12.6±2.33	13±3.16	19.14±2.52
4.	C 591	26.8±3.06	28.2±1.08	14.6±0.49	22.9±2.5
5.	HD 2967	19.4±2.42	18.3±1.70	15.2±2.64	14.4±1.02
6.	C 518	25.6±5.64	15.8±1.01	12.4±1.62	14.7±0.50
7.	C 306	28.2±3.16	28.2±1.93	14±0.89	15±0.82
8.	PBW 550	20.2±1.33	30.7±0.52	15.4±3.14	15±0.74

All data are means ± SD of each group. ±SD (standard deviation)

Table 4: Plant height data of eight wheat genotypes

S.No.	Genotype	Plant Height (Cm)
1.	HD 3086	103±2.00
2.	C 323	125±1.41
3.	WH 1105	100±3.40
4.	C 591	128±2.82
5.	HD 2967	102±3.56
6.	C 518	124±2.82
7.	C 306	128±2.72
8.	PBW 550	105±1.41

All data are means ± SD of each group. ±SD (standard deviation)

CONCLUSION

The present study clearly demonstrated the expression of the semidwarfing genes Rht-B1b and Rht-D1b in few Indian wheat cultivars and also proved that these semidwarfing genes are Gibberellic acid insensitive as their ability to confer dwarfism is not affected even upon exogenous GA_3 application.

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