



Identification of genotypes possessing GA responsive reduced height genes in bread wheat (*Triticum aestivum*)

PRAVEEN K YADAV¹, MONIKA A JOSHI², RAJBIR YADAV³ and MANJEET SINGH⁴

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

Received: 10 January 2019; Accepted: 25 January 2019

ABSTRACT

The present study was conducted during years 2017-18 and 2018-19 involving 60 wheat genotypes including advance lines and released varieties. Based on the coleoptile length, these were categorised for coleoptile length as short (2.5-4.5 cm), medium (4.6-6.5 cm) and long (6.6-9 cm) respectively. Small and medium coleoptile length genotypes had comparable plant height (100.07cm and 99.29 cm, respectively) whereas, longer coleoptile genotypes were in general taller (103.78cm) numerically and for each class, the speed of germination was almost similar. A set of linked SSRs markers was used to screen the genotypes for molecular characterization based on GA responsive genes. In wheat, longer coleoptile length with reduced height is primarily controlled by GA responsive reduced height genes like *Rht8*, *Rht12* etc., therefore, the material was screened to identify genotypes possessing GA responsive reduced height genes with available molecular markers. Molecular screening for *Rht* genes revealed five *Rht8* positive genotypes having longer coleoptile length and good seedling vigour. Thus, *Rht8* genes had positive effect on coleoptile length and had no detrimental effect on root length of seedling and provided better field emergence in field. Hence, these genotypes can be used for the development of variety for dryland agriculture and underconservation agriculture practices.

Key words: Coleoptile length, Dryland agriculture, Reduced height genes, SSR markers, Seedling traits

Wheat (*Triticum aestivum* L.) yield remained almost stagnant in the last two decades and approaches for sustainability of wheat based cropping production system mainly the rice-wheat cropping system are needed. Sustainability is a global issue and mainly revolves around the issues of soil degradation affecting about 35% of the earth's soil surface and ground water depletion due to excessive irrigation. Beside these issues, climate change is projected as biggest threat to food security at global level. Climate change models, as developed by the Intergovernmental Panel on Climate Change (IPCC), predict that temperatures in India are likely to rise by 3-4°C by the end of the 21st century. Reducing profit and production environment emphasize the need to think about change in the production methods hence, conservation agriculture practices like zero-tillage and residue retention, which offer many advantages, are immediately needed (Chan 2001 and Schmidt *et al.* 2003). The total land area of India is 329 million ha of which 144 million ha is arable land. Of these

94 million ha fall under dry lands, amounting to 33% of wheat production. Enhancing the production of limited irrigation areas seems an attractive way to increase the productivity and production of wheat by introduction of alternate cropping system across rice-wheat areas. In the dryland area, upper soil moisture is depleted very rapidly after the sowing due to higher rate of evaporation. Hence depth of sowing in these areas becomes an important factor for field emergence in semi dwarf varieties of wheat. Thus the coleoptile length of the seedling becomes an important feature for the proper field emergence (Mohan *et al.* 2013). Similarly, moisture depletion takes place very rapidly with very early sowing of wheat due to presence of high temperature at that time. Thus higher depth of sowing facilitated by longer coleoptile length is of utmost importance for uniform establishment of crop for getting the higher productivity. In wheat, longer coleoptile length with reduced height is controlled by GA responsive reduced height genes like *Rht8*, *Rht12* etc., therefore, these genes can be incorporated into desired one for better establishment of crop plants under target environment (Pandey *et al.* 2015). In lieu of the above, the present study was undertaken to screen the wheat germplasm, including advance lines for longer coleoptile length genotypes; and to identify genotypes possessing GA responsive reduced height genes with available molecular markers.

¹Ph D Scholar (iaripky@gmail.com), ²Principal Scientist (monikakshat622@gmail.com), Division of Seed Science and Technology, ³Principal Scientist (rajbiryadav@yahoo.com.com); ⁴Scientist (manjeetbhu615@gmail.com), Division of Genetics.

MATERIALS AND METHODS

Experimental material comprised 60 genotypes obtained from Division of Genetics, Indian Agricultural Research Institute, New Delhi and was planted during *rabi* season of 2017-18 and 2018-19. The list of advance lines (denoted by CLY numbers; Cereal Lab Yielding), along with their pedigree details along with selected released varieties is given in Table 1. The plant height was recorded from the base to the tip of the spike after completion of heading in ten randomly selected plants per replication and mean plant height was expressed in cm. For measuring the coleoptile length, 25 seeds were kept on a moist germination paper with germ end down having 1 cm markings on either side of the central line, and kept in upright position at 20°C in dark and observation was taken on 10th day. Based on the observations, the genotypes were divided into 3 categories of coleoptile length namely; short (2.5-4.5 cm), medium (4.6-6.5 cm) and long (6.6-9 cm) (Table 1). Speed of germination was calculated by the formula as suggested by the Maguire (1962).

For measuring shoot length, ten normal seedlings were selected randomly for measuring shoot length and expressed in centimetres (cm). To screen the genotypes for GA responsive genes, a set of SSRs markers was used for molecular characterization of 40 wheat genotypes of medium and long coleoptile length. The SSR markers utilized in the present study were gene specific primer for *Rht* genes. Molecular analysis was undertaken by following the sequential steps via established protocols for leaf sampling, DNA isolation, DNA purification and quantification, and polymerase chain reaction. Polymerase chain reaction (PCR) is used to selectively amplify *in vitro*, a specific segment of the DNA to a billion fold (Mullis *et al.* 1986). The most essential requirement of PCR is the availability of a pair of short (typically 20-25 nucleotides) primers having sequence complementary to either end of the target DNA segment (called template DNA). The separation of PCR amplified products was done on 3.5% metaphore agarose gel. After separation of amplified products of each reaction on 3.0% metaphor agarose gel, it was photographed using a Gel Documentation System, under equal magnification. Scoring was done manually for each of the gel sections. Allele's sizes were determined based on the position of the bands relative to the ladder. Band patterns for each of the microsatellite markers were recorded for each genotype by assigning size of band in term of base pairs based on the ladder of minimum 50bp size. Finally alleles were numbered as 'a1', 'a2' etc. sequentially from the largest to the smallest-sized bands to analyze their frequencies. For each allele, differences in genotypes were indicated by scoring (1) for presence or (0) for absence of band. Any band thought to be an artifact or diffused bands were considered as 'missing data. These missing data were designated as '9' (in comparison with '1' for presence of a band and '0' for absence of a band). 'Null' allele for any specific marker in a genotype was again considered as absence of band (designated as '0') clearly indicating the absence of primer binding site, after re-runs

with specific check. The re-runs facilitated the confirmation of allele scoring in various genotypes. Molecular marker data were entered directly into Excel spreadsheet, with the microsatellite alleles under rows and the genotypes under columns.

RESULTS AND DISCUSSION

The plant height for all the genotypes was recorded and list of better performing ones with good speed of germination are highlighted with an asterisk sign and are presented (Table 1). In addition, the coleoptile length of all the 60 genotypes was also recorded and categorised as short (2.5-4.5 cm), medium (4.6-6.5 cm) and long (6.6-9 cm) (Table 1).

A perusal of results revealed that genotypes with small and medium coleoptile length had comparable plant height (100.07 cm and 99.29 cm, respectively), whereas longer coleoptile genotypes were taller with 103.78 cm plant height. Further, no definite relation could be established between speed of germination and genotypes of three different classes, i.e. short (2.5-4.5 cm), medium (4.6-6.5 cm) and long (6.6-9 cm) coleoptile length genotypes. For each class, the speed of germination was 38.70, 38.20 and 39.60 respectively (Table 1). The speed of germination is mainly dependent on the radical appearance which is a part of root initials, and no effect of *GARRht* genes on root length has been reported till date. Hence this explains the possible cause for non-existence of any definite relation. The observations recorded from the study of coleoptile length and shoot length, clearly emphasized that coleoptile length was directly proportional to seedling shoot length, i.e. short (2.5-4.5 cm), medium (4.6-6.5 cm) and long (6.6-9 cm) coleoptile classes had on an average 7.12 cm, 8.87 cm, and 12.60 cm shoot lengths respectively (Table 1). This establishes that long coleoptile genotypes had an added advantage of better photosynthesis and dry matter accumulation over the short and medium coleoptile genotypes during early developmental stages and helps in better field establishment.

To screen the genotypes for GA responsive genes, a set of SSRs markers, i.e. *Rht4*, *Rht5* and *Rht 8* were selected because these genes had tight linkage with these markers and hence, were used for molecular characterization of the selected 40 genotypes of medium (4.6-6.5 cm) and long (6.6-9 cm) coleoptile length. The SSR markers utilized in the present study were gene specific primer for *Rht* genes (Table 2). Among the 40 genotypes, there were only five genotypes which were *Rht8* positive (Table 2) and in none of the genotypes *Rht4*, and *Rht5* expression was found.

Potential *Rht8* positive genotypes that had long coleoptiles length and also had higher values of shoot length and speed of germination might be taken as source and also these traits will be considered as selection criterion for shortlisting genotypes suitable for drought environments. The study revealed that *Rht8* genes had positive effect on coleoptile length and had no detrimental effect on root length of seedling and thus provided better chance for early

Table 1 Categorisation of experimental material into short, medium and long coleoptile length and other morphological-cum-seedling traits

Genotype	Pedigree	Coleoptile length (cm)	Plant height (cm)	Speed of germination	Shoot length (cm)
<i>Short coleoptile length genotypes</i>					
CLY1642	7 EBWYT 504	3.64	97.67	38.75	6.80
CLY1647	HD2874/HD2967//43rd IBWSN 1148	3.82	102.33	38.17	6.60
CLY1648*	HD2874/HD2967//43rd IBWSN 1148	3.78	99.33	40.17	7.24
CLY1649*	HD2874/HD2967//43rd IBWSN 1087	3.80	106.83	41.00	7.44
CLY1650	HD2874/HD2967//43rd IBWSN 1087	3.50	101.50	37.33	6.58
CLY1652	10 SBWON-27//PBW 343/DW571	3.52	103.33	37.67	6.92
CLY1653*	31ESWYT-113//DW1272/HP1731	3.44	100.67	39.83	6.82
CLY1656	31ESWYT-113//DW1272/HP1731	3.76	95.67	38.60	7.26
CLY1659	31ESWYT-147/3/HW5028//HD2432/DW1309	4.56	102.00	38.25	7.74
CLY1662	18 HRWYT 214/18HRWYT-229	3.56	99.33	38.42	7.62
CLY1664	18 HRWYT 214/18HRWYT-229	3.90	97.50	36.42	6.82
CLY1670*	HD 2824/VL804//PBW532/UP2425	3.58	98.50	40.75	7.00
CLY1679	EBWYT 60	3.54	103.67	36.42	7.00
CLY1684	Recombinant inbred line (RILs)	3.98	97.00	37.50	7.30
CLY1686	CL1449/PBW343//WL412/Vei/Koel/3/Pes/Mc-II	3.48	100.17	39.08	7.80
CLY1698*	31 ESWYT 138/CSW23	3.80	97.33	39.90	7.00
CLY1708	PBW343/CL1538//HD2932/HD2189	3.98	110.50	39.42	7.04
HD3086		3.60	100.67	38.17	7.72
HD 3117		4.26	111.83	38.58	8.24
HD 2967		3.86	73.33	39.67	7.38
Mean		3.77	100.07	38.70	7.12
<i>Medium coleoptile length genotypes</i>					
CLY1601	CL2596/K9451/CL882//HD2009	5.44	91.17	37.75	9.08
CLY1610*	CL2596/K9451//CL882//HD2009	5.62	108.83	40.00	9.56
CLY1622	C-32 SAWSN 327	5.60	100.67	38.00	9.92
CLY1632*	HD2953/HS365	5.30	97.17	40.08	9.16
CLY1634	SAWSN 3094	5.48	100.50	37.08	9.00
CLY1635	SAWSN 3097	5.66	96.00	35.17	7.98
CLY1638	18 HRWYT 214	5.44	101.67	38.00	9.44
CLY1651*	HD2874/HD2967//43rd IBWSN 1087	4.86	87.67	39.92	7.78
CLY1657	31ESWYT-147/3/HW5028//HD2432/DW1309	4.96	105.33	36.00	9.30
CLY1676	SAWSN 3194	5.42	110.50	38.58	9.26
CLY1677	CSISA-HT-EM-37	5.32	99.83	38.17	9.06
CLY1678	SRRSN 6083	5.26	101.00	34.25	8.68
CLY1680	EBWYT 98	5.26	99.67	38.67	8.40
CLY1681	EBWYT 81	4.96	86.00	38.08	8.46
CLY1692	31 ESWYT 135/CSW23	5.26	110.67	38.75	9.20
CLY1693*	31 ESWYT 135/CSW23	5.04	95.67	39.17	9.36
CLY1695	31 ESWYT 135//HD2329/WR544/PBW343/ NW3041	4.82	104.83	39.00	8.70
CLY1701*	31 ESWYT 138//PBW343/PH137/MC-II	4.82	78.83	40.42	7.14

Cond.

Table 1 (Concluded)

Genotype	Pedigree	Coleoptile length (cm)	Plant height (cm)	Speed of germination	Shoot length (cm)
CLY1707	31 ESWYT 138/CSW30	5.12	107.33	38.90	8.78
HD2329		6.10	102.50	38.18	9.26
Mean		5.29	99.29	38.20	8.87
<i>Long coleoptile length genotypes</i>					
CLY1606*	CL1633/ CNo. 601// CL1633/ CNo. 601	7.90	110.20	40.42	10.88
CLY1611	HD2967/NIVT-1A(3A)	8.52	113.17	40.17	12.64
CLY1612	SAWYT-319(06-07)	7.90	102.67	36.33	12.84
CLY1613	CP264//HD2839/ HD2329	8.46	102.67	39.00	13.48
CLY1615	HD2329/HDK-10//CBW38/WR541	8.34	98.50	39.58	11.62
CLY1617*	IBWSN70//IBWSN 1053	8.42	103.33	40.83	12.64
CLY1621	C-32 SAWSN 179	7.40	101.83	40.17	11.88
CLY1630	HD 2878/HD29	8.36	107.33	38.42	13.24
CLY1636	EBWYT 21	7.96	100.67	38.83	12.84
CLY1641	28 SAWSN 3157	8.22	97.67	40.25	12.30
CLY1644	VL 616 (2) Inqulab/Kundan	8.90	109.17	37.83	12.72
CLY1661	18 HRWYT 214/18HRWYT-229	8.30	94.83	40.34	12.82
CLY1668	18 HRWYT 222//VL849/UP2571	7.82	105.67	39.83	12.82
CLY1683*	SAWYT-331	7.68	102.17	41.58	13.02
CLY1700*	31 ESWYT 138//PBW343/PH137/MC-II	7.36	92.83	41.58	11.60
CLY1706	31 ESWYT 138/CSW30	8.52	100.50	38.25	13.64
NP4*		7.66	105.33	42.75	12.32
NP818		7.96	107.17	39.92	12.84
C 306		7.96	121.45	39.33	13.12
HDCSW18		7.42	98.45	36.58	12.84
Mean		8.05	103.78	39.60	12.60
CD (P=0.05)		0.136847	0.836571	0.798616	0.337539

field emergence in drier environments. Similar results were found by Botwright *et al.* (2001), Rebetzke *et al.* (2004), Robbins (2009), Liatukas and Ruzgas (2010), Gesperini *et al.* (2012) and Pandey *et al.* (2015). The genotypes with longer coleoptile length are known to have better field emergence, good root biomass characteristics and hence very suitable for the development of variety for dryland agriculture

Table 2 Three SSR molecular markers used to screen *Rht4*, *Rht5* and *Rht8* genes

Gene	Chromosome locus	Linked marker	Positive genotypes
Rht4	2B	Xwmc 317	-
Rht5	3B	Xbarc 102	-
Rht8	2D	Xgwm 261	CLY 1621, CLY 1630, CLY 1636, CLY 1641, CLY 1661

and modern cultivation practices such as conservation agriculture. Grover *et al.* (2018) selected different genotypic combinations for *Rht1* and *Rht8* genes and reported additive effect of genes as double dwarfs which showed significant reduction in coleoptile length and seedling shoot length. Similar results were reported by Tang *et al.* (2009) where they concluded that GA₃-insensitive dwarfing genes (*Rht-B1b* and *Rht-D1b*) are not suitable for the wheat improvement in dryland because these two genes reduce both plant height and coleoptile length. However, GAR dwarfing gene (*Rht8*) is a relatively competent candidate for the wheat improvement under dryland conditions since it significantly reduced the plant height of wheat, but had less effect on the coleoptile length.

Hence, it is concluded that *Rht8* positive genotypes had longer coleoptile length and better speed of germination and therefore, have better adaptability in drylands. So these genotypes are recommended to be utilised for development of new genotypes for dryland

conservation agricultural practices.

REFERENCES

- Botwright T, Rebetzke G, Condon T and Richards R. 2001. The effect of *rht* genotype and temperature on coleoptile growth and dry matter partitioning in young wheat seedlings. *Functional Plant Biology* **28**(5): 417–23.
- Chan K Y. 2001. An overview of some tillage impact on the earthworm population abundance and diversity implication for functioning in soil. *Soil and Tillage Research* **57**:197–1.
- Gasparini D, Greenland A, Hedden P, Dreos R, Harwood W and Griffiths S. 2012. Genetic and physiological analysis of *Rht8* in bread wheat: an alternative source of semi-dwarfism with a reduced sensitivity to brassinosteroids. *Journal of experimental botany* **63**(12): 4419.
- Grover G, Sharma A, Gill H S, Srivastava P and Bains N S. 2018. *Rht8* gene as an alternative dwarfing gene in elite Indian spring wheat cultivars. *PloS one* **13**(6): e0199330.
- Liatukas Ž and Ruzgas V. 2011. Coleoptile length and plant height of modern tall and semi-dwarf European winter wheat varieties. *Acta Societatis Botanicorum Poloniae* **80**(3): 197.
- Maguire J D. 1962. Speed of germination—Aid in selection and evaluation for seedling emergence and vigor I. *Crop science* **2**(2): 176–7.
- Mohan A, Schillinger W F and Gill K S. 2013. Wheat seedling emergence from deep planting depths and its relationship with coleoptile length. *PLoS One* **8**(9): e73314.
- Mullis K, Faloona F, Scharf S, Saiki R K, Horn G T and Erlich H. 1986. Specific enzymatic amplification of DNA *in vitro*: the polymerase chain reaction. *Cold Spring Harbor Symposia on Quantitative Biology* **51**:263–73.
- Tang Na, Jiang Y, He B R and Hu Y G. 2009. The effects of dwarfing genes (*Rht-B1b*, *Rht-D1b*, and *Rht8*) with different sensitivity to GA3 on the coleoptile length and plant height of wheat. *Agricultural Sciences in China* **8**(9): 1028–38.
- Pandey M, Singh A K, DePauw R M, Bokore F E, Ellouze W, Knox R E and Cuthbert R D. 2015. Coleoptile length, gibberellin sensitivity, and plant height variation of durum wheat in Canada. *Canadian Journal of Plant Science* **95**(6): 1259–64.
- Rebetzke G J, Richards R A, Sirault X R R and Morrison A D. 2004. Genetic analysis of coleoptile length and diameter in wheat. *Australian Journal of Agricultural Research* **55**(7):733–43.
- Robbins A M. 2009. Dwarfing genes in spring wheat: an agronomic comparison of *Rht-B1*, *Rht-D1*, and *Rht8* (Doctoral dissertation, Montana State University-Bozeman, College of Agriculture).
- Schmidt O, Clement R O and Donaldson G. 2003. Why do cereal-legume intercrops support large earthworm populations? *Applied Soil Ecology* **22**:181–90.