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Screening for *Rht* genes in Wheat (*Triticum aestivum L.*) for Development of Varieties Suitable for Dry land Agriculture

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Abstract—*The total land area of India is 329 million hectares of which 144 million hectares is arable land. Of this, 94 million hectares falls under dry lands which amount to thirty three per cent of wheat production. In the dry land area, upper soil moisture is depleted very rapidly after sowing due to higher rate of evaporation. Hence, 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. In lieu of the above, the present study was conducted during year 2017-18 on 20 genotypes of long (6.6-9 cm) coleoptile length. To screen the genotypes for GA responsive genes, a set of SSRs markers was used for molecular characterization; all the SSR markers utilized in the present study were gene specific primer for Rht genes. Among the 20 genotypes there were 6 genotypes which were Rht8 positive along with 2 known test genotypes. Rht8 positive genotypes had coleoptile length in range of 7.4- 8.36 cm and also had higher values of shoot length, embryo size, surface area, root volume, number of forks, SVI I, SVI II and emergence from different sowing depths i.e. 5 cm, 7.5 cm and 10 cm. 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. These genotypes had longer coleoptile length, better field emergence, good root biomass characteristics and hence very suitable to be used for the development of variety for dryland agriculture and modern cultivation practices such as conservation agriculture.*

1. INTRODUCTION

Dryland areas contribute significantly to wheat (*Triticum aestivum L.)* production, amounting to thirty three per cent of wheat production. Enhancing the production of

dryland areas seems an attractive way to increase the productivity and production of wheat by introduction of alternate cropping system in rice-wheat areas. New production methodology like conservation agriculture can provide long term solution. 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 easily incorporated into desired one for better establishment of crop plants under target environment [1]. Hence, higher crop yield is mainly dependent on the rapid and uniform field establishment of crop in the field, which is highly influenced by the sowing depth and the ability of the seedlings to emerge from the soil. Thus the study was undertaken to screening wheat germplasm for GA responsive reduced height genes with available molecular markers.

2. MATERIALS AND METHODS

The material for the study comprised of twenty genotypes of wheat which had long (6.6-9 cm) coleoptile length (Table 1). To screen the genotypes for GA responsive genes, a set of SSRs markers was used for molecular characterization of these 20 wheat genotypes. 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. 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 50 bp size. Finally alleles were numbered as 'al', '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.

3. RESULTS AND DISCUSSION

To screen the genotypes for GA responsive genes, a set of SSRs markers was used for molecular characterization of 20 wheat genotypes of long (6.6-9 cm) coleoptile length. Details regarding the SSR markers used for present study provided in Table 2. The SSR markers utilized in the present study were gene specific primer for *Rht* genes. Among the 20 genotypes there were 6 genotypes which were *Rht8* positive along with 2 known test genotypes and in none of the genotypes *Rht4*, and *Rht5* expression was found. *Rht8* positive genotypes (Table 3) had coleoptile length in range of 7.4- 8.36 cm and also

had higher values of shoot length, embryo size surface area, root volume, number of forks, SVI I, SVI II and emergence from different sowing depths i.e. 5 cm, 7.5 cm and 10 cm.

Hence, five genotypes were found *Rht8* positive and their performances also in accordance with the above studies it was found that the coleoptile lengths of all above genotypes ranged between 7.4 cm and 8.4 cm and categorised into long coleoptile length class of genotypes. These genotypes had emergence % of 80- 90 % except one having 75% emergence from deep sowing of 10 cm depths. This study revealed that 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. Similar results were found by several workers [2-6].

Hence, these genotypes have longer coleoptile length, better field emergence, good root biomass characteristics and hence very suitable to be used for the development of variety for dryland agriculture and modern cultivation practices such as conservation agriculture. Similar results were reported by Na et al., *(*2009) [7], they concluded that GA_3-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.

Table 1: List of genotypes

S. No.	Name	Pedigree
1	CLY1606	CL1633/ CNo. 601// CL1633/ CNo. 601
2	CLY1611	HD2967/NIVT-1A(3A)
3	CLY1612	SAWYT-319(06-07)
4	CLY1613	CP264//HD2839/ HD2329
5	CLY1615	HD2329/HDK-10//CBW38/WR541
6	CLY1617	IBWSN70//IBWSN 1053
7	CLY1621	C-32 SAWSN 179
8	CLY1630	HD 2878/HD29
9	CLY1636	EBWYT 21
10	CLY1641	28 SAWSN 3157
11	CLY1644	VL 616 (2) Inqulab/Kundan
12	CLY1661	18 HRWYT 214/18HRWYT-229
13	CLY1668	18HRWYT 222//VL849/UP2571
14	CLY1683	SAWYT-331
15	CLY1700	31 ESWYT 138//PBW343/PH137/MC-II
16	CLY1706	31 ESWYT 138/CSW30
17	NP4	
18	NP818	
19	C 306	
20	HDCSW18	

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

S. No.	Gene	Chromosome locus	Linked marker
1.	Rht4	2B	Xwmc 317
2.	Rht5	3B	Xbarc 102
3.	Rht8	2D	Xgwm 261

Table 3: Rht8 positive genotypes and their pedigree

CLY NO.	Pedigree
CLY 1621	C-32 SAWSN 179
CLY 1630	HD 2878/HD29
CLY 1636	EBWYT 21
CLY 1641	28 SAWSN 3157
CLY 1661	18 HRWYT 214/18HRWYT-229

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