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Research Article

Agronomic association of *Vrn, Ppd, Rht* genes and identified QTLs under contrasting tillage conditions in wheat

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Abstract

Improved varieties have played important role in higher yield realization in tandem with improved management practices. Conservation agriculture as an improved environment for higher production requires adaptive variety to further the yield gain. Higher effect of environment along with equally important G×E makes selection on the basis of phenotypic traits more risky and less rewarding. Molecular markers with lesser effect of environment and independent of stage and condition for their expression present attractive option to breeders to improve selection efficiency. Various *Vrn, Ppd* and *Rht* genes along with number of QTLs identified by researchers have adaptive role in wheat. Genotypes showing differential adaptiveness were grown under three contrasting environments generated by different tillage-sowing methods. Gene specific markers were scored and genotypes were grouped as per markers profile. Grouping on the basis of markers was correlated with phenotypic grouping, though not so strongly, under different environments, picking conservation agriculture adaptive genotypes under one node and genotypes adapted for conventional tillage in another node. Contrasting pool created by identified QTLs under its adaptive growing environment does not show any differentiating ability and therefore requires further validation before use in breeding programme.

Keywords: Wheat, conservation agriculture, molecular markers, QTLs.

1. Introduction

The gain in productivity achieved in the past through improved varieties to produce more within limit set of environment and improved management practices making the growing environment and supply of resources (plant nutrients and water) more favorable for the crop may not necessarily sustainable in the coming times and may require further modification and changes in both. These two fundamental drivers of growth which appears to be seamless in fact have grown in tandem over the years (cook, 2006). Stagnating yield in major crops like wheat and rice, need innovative solution on both management and varietal development front. Evidences are now accumulating which suggest direct seeded cropping system has much higher yield potential because of more water availability, better nutrient recycling and improved soil health. Conservation agriculture though basically strives for sustainable productivity, quality and economic viability with soil and water preservation (Jordan and Hutcheon, 1997; El Titi, 2003; SOWAP, 2006) but still, the potential gain of wheat based cropping system under CA is not known and may still be more higher and can be achieved through more changes in management practices and by breeding better adapted varieties. As the environment under CA shifts due to altered soil structure, different host-pathogen interaction, better availability of moisture, the genotypes developed on conventional changes probably may not harness the improved environment (Duvick, 1990 and Trethowan *et al*., 2005). Genetic adaptation under CA have comparatively been lesser studied event than agronomy (Liebman and Davis, 2000; Cook, 2006; Mahmood *et al*., 2009).

Effort to develop better adapted varieties for conservation agriculture, require revisit to some of the characters like

coleoptiles length and its relationship with *Rht* genes, duration of developmental stages and their relationship with *Vrn* genes. Coleoptile take the growing wheat shoot from the depth where the seed has been placed to the top of soil and a failure to do so results in poor stand establishment. Deep seeding is preferred under the condition of limited moisture and uneven seed bed. In conservation agriculture, seed bed is greatly uneven and therefore requires deeper placement of seed for uniform stand establishment. The necessity of application of non-selective herbicide like glyphosate before crop seeding under conservation agriculture and to avoid the contact with germinating seedling with herbicide, make the deep seeding imperative. Length of coleoptiles is controlled by various factor including *Rht* gene (Rebetzke *et al*., 1999) and soil and ambient temperature (Radford 1987, Rebetzke *et al*., 1999) and seed mass (Addae and Pearson 1992). Majority of the present day cultivar in wheat in India carry *Rht* 1 and *Rht* 2 gene which has limited coleoptile length. The other Rht genes like *Rht* 8, 12 are sensitive to gibberellin and produces longer coleoptile but overall moderate plant height. Similarly, wheat under conservation agriculture grows under more favourable environment and therefore needs further optimisation of timing and duration of various development phases as optimisation of development phase is more crucial for adaptation for a particular environment (Richards, 1991; Worland, 1996). Genetics of flowering time is extensively studied but little known about preflowering time. Extension of stem elongation phase [SE; from terminal spikelet initiation (TS) to anthesis] without altering the time to anthesis mean for further increase in yield potential (Slafer *et al*., 2001).Probably may be more rewarding under CA than under conventional tillage because of better environment available for plant growth. The rationale for this proposition is that the length of the stem elongation phaseis a major yield determinant as the number of fertile florets at anthesis, which determines the final number of grains, set during this phase (Kirby, 1988; Slafer and Rawson,1994; Fischer, 2007; Miralles and Slafer, 2007).There are reports which indicate that different pre-heading phases could be changed without modifying time to anthesis by exploiting available variability for vernalization, photoperiod, and response to temperature (Slafer, 1996; Miralles and Richards, 2000; Gonzalez *et al*., 2002). In wheat, candidate genes have been identified for photoperiod (Scarth and Law, 1983; Mohler *et al*., 2004) and vernalization response (*Vrn*-A1, *Vrn*-B1, and *Vrn*-D1) (Law *et al*., 1976; Worland*et al*., 1987; Galiba *et al*., 1995) and are well identified.

2. Materials and methods

The experimental material for the present investigation comprised of 42 wheat genotypes (Table1) including released and pre-released advance lines representing a range of phenotypic variability and genetically diversity. A set of 27 Simple Sequence Repeats (SSR) markers were used for QTL validation which were identified previously, however, only 15 SSR markers showed polymorphism. These include'Xgwm', 'Xwmc' 'Xcfa', cfd markers and gene specific primer for *Vrn*, *Rht* and *Ppd* genes. Details of SSR markersand gene specific primer along with sequences of the forward and reverse primers and annealing temperature are given on the grain genes wheat microsatellite consortium (http://wheat.pw. usda.gov.).

2.1 DNA extraction and PCR analysis: Total genomic DNA was isolated from 5 gm of fresh young leaf tissue, collected from five, one-month old, bulked leaf samples following the cetyl-tri-methyl ammonium bromide (CTAB) method. DNA was precipitated with chilled iso-propanol and DNA pellet was rinsed with 70 per cent ethanol for 10-15 minutes to remove excess CTAB. The pellet was dried at room temperature overnight and dissolved in TE buffer (pH=8). The purified DNA was quantified on 0.8 per cent agrose gel along with a standard containing 100 ng/mL genomic λ DNA. The total genomic DNA was diluted to 20 ng/µl for use in PCR analysis.

A set of reported 27 Simple Sequence repeat (SSR) markers were used out of which 15 were found to be polymorphic among these 7 markers were tillage specific. Gene specific primers for *Vrn*, *Rht* and *Ppd* genes are used to know their presence and absence. The micro satellite markers have previously been developed and their map position, chromosomal location, and repeat motifs can be found in the wheat micro satellite consortium (http://wheat.pw. usda.gov/). PCR amplification was performed with initial denaturation at 94°C for 3 min followed by 35 cycles of 94°C for 1 min, 50°C, 55°C or 60°C (depending upon the primer) for 1 min, 72°C for 2 min, and a final extension at 72°C for 10 min before cooling at 4°C. Amplification products were resolved on 3 per cent metaphor agarose gel and DNA bands were visualized by staining with ethidium bromide. Data were analysed using SPSS 16.0, simple *t* test for difference of mean among the marker genotype and the trait value. Analysis of *'t'* value observed compared against the tabulated *'t'* value. Gene specific marker was scored for presence and absence.

3. Result and discussion

Effort to develop better adapted varieties for conservation agriculture, require revisit to some of the characters like coleoptiles length and its relationship with *Rht* genes, duration of developmental stages and their relationship with *Vrn* genes and *Ppd* genes. In the present context for association of these genes with particular production environment a Dendrogram (Figure 1) was constructed. From this dendrogram it is inferred that some of the zero-tillage adapted varieties like HD 3117, CSW15, CSW 18, CSW 25 are in the one cluster. Some of the other varieties adapted to conventional tillage condition viz; CSW 3, CTFB 4567, CTFB 4550 and PBW 550 are in one cluster.

Table 1. Parentage of genotypes used in the genotype x management interaction studies

Genotypes	Genotype codes	Pedigree
HD 3115	G1	HW 5028/HD 2285
HD 3117	G2	HD 2733/HD 2824// DW 127
CSW 1	G3	HD 2329/WR 562
CSW 2	G4	CL 1579/HD 2329
CSW 3	G5	WL 462/VE//KOE1//3/ Pastro/MC 11
CSW 4	G6	Kauz//Altar 84/AOS/3/ Milan
CSW 5	G7	PBW 502/HW 5028// CL 1673
CSW 7	G8	CBW 38/WR 541
CSW 10	G9	HD 2377/HD 2329
CSW 13	G10	HW 1083/CL 14820
CSW 15	G11	CL 1449/PBW 343
CSW 16	G12	CL 1449/PBW 343
CSW 18	G13	PBW 343/CL 1538
CSW 21	G14	UP 2586/PBW 343
CSW 22	G15	WR 567/HD 2329
CSW 23	G16	PBW 343/CL 1538
CSW 24	G17	PBW 343/HUW 567
CSW 25	G18	PBW343/4/CHOIX/Star/3/ HEI/3*CNO79//2*SERI
CSW 26	G19	UP 2425/CL 1482
CSW 27	G20	HW 5055/WR 196
CSW 28	G21	HW 5015/HD 2643
CSW 29	G22	HD 2898/MC 10
CSW 30	G23	HD 2687/CL 1116
CSW 31	G24	CL 1449/HUW 585
CSW 32	G25	PBW 343/HUW 567
CSW 33	G26	HD 2687/PBW 498
CSW 34	G27	HW 5015/LOK 3397
CSW 35	G28	HW 5015/KUNDAN
CSW 36	G29	PBW 343/PH 137//MC 11
CTRB 1666	G30	UP 2425/CL 1461
CTRB 1667	G31	UP 2425/UP 2626
CTRB 1813	G32	HW 5015/HD 2643//PBW 343
CTRB 1816	G33	UP 2586/K 9107
CTRB 1817	G34	HD 2329/HUW 585
CTFB 4550	G35	VL 849/NBP 1609
CTFB 4565	G36	DW 1293/CL 1525
CTFB 4566	G37	DW 1506/UP 2632
CTFB 4567	G38	HD 2733/HD 2329
CTFB 4639	G39	PBW 502/HW 5028// CL 1673
DBW 17	G40	CMH 79A95/3*CNO79// RAJ 3777
PBW 550	G41	WH 594/RAJ 3858//W 485
HD 2967	G42	ALD/COC//URES/HD 2160 M// HD2278

Some of genotypes adapted to freshly prepared raised bed are in the same cluster these are CSW 36, and CTRB 1813. When search for particular gene combination was made for each of these groupings, HD 3117 has the combination of *vrn*A, *Vrn*B, *vrn*D, *Rht*B1b, *Rht*8b, *Rht*12 tall, although in case of CSW 18 and 23 *Vrn*D is present. For group CSW 3, *vrn*A, *Vrn*B, *Vrn*D, *Rht*B1b, *Rht*D1b, *Rht*8b and *Rht*12t all but for PBW 550 *vrn*B is recessive. As compared to the previous group difference for *Rht*D1b is present in this group which is absent in the latter. While for CSW 36 and CTRB 1813 group difference exists from both the groups by containing recessive *Vrn*B. These combinations may be a cause for the adaptation to different tillage condition. But accurate conclusion can be only made by studying the effect of these genes through isogenic lines, because genetic background for each line is different so we cannot accurately find the difference on the ground of only these few genes. As the phenotype is the consequence of the sequential expression of the set of genes, so for future line of work, such experiments need to be conducted by use of the isogenic lines to draw conclusion.

Identified QTL may be integrated in the breeding program by analyzing its presence in the elite line. The QTL analysis identified some chromosomal regions of significance for trait expression under different environments for various traits, like yield, days to heading, plant height and NDVI. While numerous QTL intervals were found only those significant at $P > 0.05$ in at least one tillage treatment and those associated with specific tillage practice are used for the testing of significance of mean. Significant yields were observed between the contrasting tillage system and among genotypes. Those QTLs noted in both tillage treatments are therefore unlikely to be tillage regime specific and are not used for the analysis of t-test.

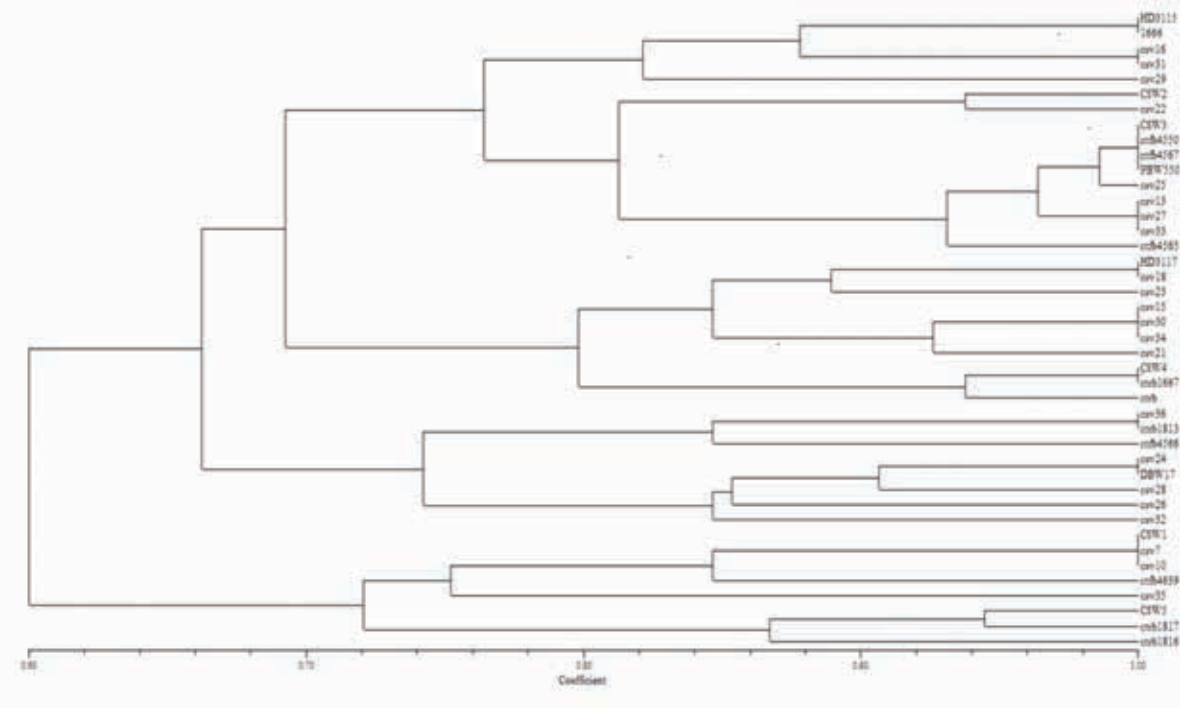

Fig 1. Dendrogram based on the genes *Vrn*, *Rht* and *Ppd*

Table 2. List of markers, their profile and gene effect in wheat

Trait	Marker interval	Chromosome	No.	Additive effect
Yield	Gwm268/wPt3475	1B	8	Plymorphic
	Gwm140/wPt1313	1B	10	Monomorphic
	Wmc 216/cfd19	1D	10	Monomorphic / polymorphic
	Cfd 44/wPt3728	2D	9	Monomorphic
	Gwm484/wmc27	2D	9	Polymorphic
	Cfa 2115/wPt1370	5A	14	Monomorphic
	Wmc99/wPt2373	5B	12	Monomorphic
Heading	Gwm275/wPt3114	2A	8	Polymorphic
	Gwm95/wPt3114	2A	10	Polymorphic
	Gwm102/wPt0298	2D	9	Polymorphic
	Cfd19/wmc215	5D	14	Polymorphic / monomorphic
	Cfd19/cfd7	5D	17	Polymorphic / polymorphic
	Wmc807/wpt7063	6A	9	Polymorphic
	Cfd190/gwm219	6B	9	Polymorphic / monomorphic
Height	Gwm95/wPt3114	2A	16	Polymorphic
	Cfd44/wPt9797	2D	9	monomorphic
	Wmc106/wmc48	4A	11	Polymorphic / polymorphic
	Wmc807/wPT6063	6A	16	Polymorphic
NDVI-1	Wmc141/wmc617	4B	9	Polymorphic / polymorphic
NDVI-2	Wmc106	4A	9	Polymorphic /

Several tillage specific QTLs for yield, plant height, heading and NDVI were observed to be polymorphic in our test material and few are monomorphic (Table 2). All these polymorphic markers were scored and based on the marker genotype different groups were formed and these groups were subjected to t test. None of the observed QTLs was significant in our condition as can be seen in the *'t'* test (Table 3) for each tillage specific marker. One reason for this may be the difference of the background in which these QTLs were identified are different from the Indian germplasm background, other reason for its non-significance may be the cultivars in which these were identified were adapted to the rainfed condition; while our genotypes were selected from the normal-sown fully irrigated condition which is in tune with the report by that no significant QTLs were observed in their material also during the wet season (Trethowan*et al*., 2012).

Table 3. Value of *'t'*-test for identified QTL

Marker	*t*–value	Specificity	Significance
M484	0.45883	ZT	Non-significant
Gwm275	0.340376	ZT	Non-significant
Wmc807	0.357791	ZT	Non-significant
GWM95	0.328296	ZT	Non-significant
Cfd190	0.274387	ZT	Non-significant
Gwm268	0.077275	CT	Non-significant
Cfd19	0.092856	CT	Non-significant

Therefore further validation of these QTLs is required using some structured mapping population. It is thus clear from this study that *Rht*, *Vrn* and *Ppd* genes are important adaptive traits. The presence of genotypes with alternate dwarfing genes like *Rht* 4, *Rht* 8 and *Rht*12 in the differentially adaptive genotypes indicates toward their role in adaptiveness. Grouping of the genotypes on the basis of gene based markers, match with phenotypic grouping to a moderate extent, under different environments, picking conservation agriculture adaptive genotypes under one node and genotypes adapted for conventional tillage in another node indicate their relevance and role in such breeding programme. Contrasting pool created by identified QTLs under its adaptive growing environment does not show any differentiating ability and therefore requires further validation before use in breeding programme.

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