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Study of Ppd-D1 gene impact on different phenotypic traits in wheat (*Triticum aestivum* L.)

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Abstract
Wheat is world's most widely cultivated food crop after rice which belongs to the family Graminae (Poaceae) and the genus *Triticum.* Presence and level of expression of Ppd genes in winter wheat and spring wheat varieties can be photoperiod sensitive or insensitive. The photoperiod insensitive wheat varieties are early to flowering in short days condition as compared to their counterpart photoperiod sensitive varieties. Photoperiod sensitivity is controlled by Ppd-A1, Ppd-B1 and Ppd-D1 genes of group 2 chromosomes. Ppd-D1 gene has been identified as the major source of earliness in wheat varieties worldwide. All the 242 lines of wheat were genotyped with Ppd-D1 marker and observed 1:1 segregation among the population for the presence and absence of this gene. It was observed that presence of this gene reduced 11 traits as compared with those lines which had no Ppd-D1 gene but few traits were expressed positively by the presence of this gene. Reduction in days to heading and plant height is the common effect observed by many researchers and days to heading also affect the spot blotch severity as reflected by area under disease in this experiment. Diversity in climate is attribute of India and variability created by Ppd-D1 gene for flowering time may be exploited in breeding programme for development of varieties for divergent regions.

Keywords: Wheat, Ppd-D1 gene, photoperiod sensitivity, genotype

Introduction
The primary goal of agriculture is to provide food for every human being, and this requirement increasing day by day due to everyday increment in world human population. To feed up this large population scientist are attending their research to increase the production of main staple crops like rice and wheat. Wheat is world's most widely cultivated food crop which belongs to the family Graminae (Poaceae) and the genus *Triticum.* It is the most important food grain in the world that ranks second in total production as a cereal crop, behind maize and ahead of rice. Wheat is a temperate crop, but still sustains well under wider agro climatic conditions. Major wheat production is concentrated between 30º and 60ºN and 27º and 40ºS latitudes (Nuttenson, 1955) [15]. It is still being grown beyond these limits successfully due to its wider adoptability of diverse species, which has lead to the harvesting of this crop in one or the other parts of the world throughout the year.

It is believed that wheat developed from a type of wild grass native to the arid lands of Asia Minor. Cultivation of wheat is thought to have originated in the Euphrates Valley as early as 10,000 B.C., making it one of the world's oldest cereal crops. In the Mediterranean region, centuries before recorded history, wheat was an important food. The central Asia, Near East, Mediterranean and Ethopian regions are the world most important centre of diversity of wheat and its related species (Kundu and Nagarajaan, 1996; Perrino and Porcedo, 1990). Hindukush area is the centre of diversity of hexaploid wheat (Kundu and Nagarajan, 1996). In India, the majority of the cultivated wheat varieties belong to three main species of the genus *Triticum*, thehexaploid, *T. aestivum* L. (bread wheat), the tetraploid, *T. durum* Desf and the diploid, *T. diococcum* and *T. monococcum* Schrank; Schulb. Bread wheat accounts for approximately 95% of the wheat grown, while 4% is durum wheat and 1% is dicoccum wheat (Gupta, 2004).

Common wheat or bread wheat is a hexaploid form wheat which is found in two ecotypes: spring and winter which is characterized on the basis of requirement of their growth habit. For winter wheat it require 40^0F for several days to induce flowering. The two ecotypes of bread wheat and spring named due to their vernalization requirement are showing their response towards light are due to having many genes which encoded protein that is responsible for their sensitivity towards light and temperature and so for flowering time. Ppd-A1, Ppd-B1 and Ppd-D1 genes responsible for the photoperiod sensitivity (Kato and Yokoyana 1992; Worland *et al.* 1998; Yang *et al.*2009) [21]. The Ppd genes specify a type of pseudo response regulator (PRR) protein which involved in the activation of the photoperiod pathway leading to the induction of the Vrn-3 gene (located on 7B chromosome of wheat)

which is a homologue of the flowering locus T (FT) of A. thaliana. The wild Ppd-D1 genes becomes active only sufficient exposure of plant to LD. mutation in Ppd-B1 and Ppd-D1 have been implicated in the main in photoperiod insensitivity in wheat (Mohlar *et al.* 2004). The semi dominant Ppd-D1 a mutation has been identified as the major source of earliness in wheat varieties worldwide. Ppd-D1 allele is expressed to synthesize PRR protein and thereby cause induction of the Vrn-3 florigen, under SD and LD conditions (Baeles *et al.* 2007) [3].

It is believed that spontaneous and conscious movement of the active allele Ppd genes across land races and cultivars have been responsible for worldwide cultivation of wheat. (Pugsley 1971; Stelmakh 1993; Worland *et al.* 1998) [16,]. Understanding of the regulatory genes network underlying flowering time is a prerequisite for the designing of future wheat for the new and conventional location and sowing times, especially in the circumstance of climate change (Mathew *et al.* 2007; Rhone *et al.* 2008) [16]. The available observation on flowering time and expression of regulatory genes on certain varieties, single and multiple natural and induced mutants and RNAi construct against Ppd-D1 genes grown under a variety of controlled environments, have provided two alternative explanation for the regulation of flowering initiation.

The difference between SD flowering time and LD flowering time was unexpectedly large among the photoperiod insensitive and vernalization insensitive (Vrn-1 and Ppd-D1a) genotypes. Short fall of LD in the short day predominant season could not be the reason for the above difference, since Ppd-D1 mutation would have removed the need for LD for flowering. in the wheat plant, the principal tillers has been observed in flowering only after it has produced five or more leaves, when it is no longer juvenile and could better meet the energy requirement for the seed production.

Material and method

The present experiment was carried out at the Agriculture Research Farm of Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, during Rabi season 2014-2015.The Agriculture Research Farm, B.H.U., Varanasi is situated in South-Eastern part of the city at 25º 15.29' North latitude and 82º 59' East longitude at an height of 75.5 m above the mean sea level. The site comes under sub-tropical climate. Varanasi is subjected to extremes of weather conditions i.e. extremely hot during summer and cold during winter. Although temperature begins to rise from mid-February and attains a maximum by May-June (Mean maximum temperature is about 43.6 ºC), it starts to decrease from July onwards reaching a minimum of 5 ºC in December-January. During Rabi season 2012-13, rainfall in this area was 134.3mm, only. The average annual rainfall in this area is about 1150 mm and the mean relative humidity is 68%. Most of the precipitation is usually received during the South-West monsoon season. In this experiment, 242 germplasm lines of wheat (MNP-1).

242 diverse lines were sown with 2 replication 2 consecutive years (Rabi 2014-15,) in two replications at Agricultural Research Farm, B.H.U., Varanasi. Each germplasm was sown in two replications in paired rows of one meter length in Alfa lattice design. Line to line and plot to plot distance was 10 and 20 cm respectively.

The technique of random sampling was adopted for recording the observations on various quantitative characters of wheat. Three plants of each treatment from each replication were selected at random at the time of recording the data on 20 characters. Data of three plants were averaged entry wise in both of the replications and mean data was used for statistical analysis. Recommended package and practices were applied to raise a healthy crop.

The amplified DNA fragments generated through SSR primers were resolved through electrophoresis in 2.5 % agarose gel prepared in TAE [242 g Tris-base; 57.1ml glacial acetic acid and 100 ml 0.5 M EDTA (pH 8.0) bring final volume to 1000 ml] buffer. Ethidium bromide solution at a final concentration of0.03 ng/µl was added to the agarose solution. For electrophoresis, 15 µl of the PCR product was mixed with 2 µl of 6X loading dye (0.25% bromophenol blue in 30% glycerol) and loaded in the slot of the agarose gel. In order to determine the molecular size of the amplified products, each gel was also loaded with 1 µg DNA of a 288 bp DNA size marker (Fermentas, USA). Gel electrophoresis was performed at a constant voltage of 65 V for about 3.5 hours. Finally, the gels were visualized under a UV light source in a gel documentation system (Gel Doc^{TM} XR+, BIO-RAD, USA) and the images of amplification products were captured and stored in a computer for further analysis.

Result and discussion

Phenotypic correlation of ppd-D1 with different traits

In the present investigation (table-1) shown that Ppd-D1 gene had the highly significant and negative correlation with days to heading (-0.31**), waxiness of ear (-0.36**), physical maturity (0.208**), waxiness of peduncle (-0.16**), foliage color (-0.29**), ear length (-0.29**).Whereas Ppd-D1 exhibit the significant and negative correlation with waxiness of leaf sheath (-0.135*), Early growth habit (-0.147*) and waxiness of ear (0.124*).

On other hand Ppd-D1 exhibit the highly significant and positive correlation with AUDPC of spot blotch (0.23**), flag leaf breadth (0.15**) whereas waxiness of ear (0.136*) show significant and positive correlation.

Effect of ppd- D1 gene/marker on the phenotypic traits

It was observed that presence of this gene reduced 11 traits as compared with those lines which had no Ppd-D1 gene (Fig. 1) viz., days to heading (4.09%), 1000-grain weight (5.99%), seeds per spikes (10.36%), ear length (8.02%), days to maturity (2.63%), early growth habit (9.50%), flag leaf attitude (0.12%), flag leaf length (0.84%), waxiness of leaf sheath (4.84), waxiness of leaf blade (1.14%) and waxiness of peduncle (5.33%). But few traits were expressed positively by the presence of this gene viz., Area Under Disease Progress Curve (22.00%), auricle pigmentation (2.36%), auricle pubescence (0.24%), flag leaf breadth (3.39%), waxiness of ear (10.51%), foliage colour (1.64%), pith (3.22%) plant height (0.66%) and awn attitude (0.37%).

Table 1: Estimates of phenotypic correlation coefficient between Ppd-D1 and its related traits from 242 genotypes of wheat

	TGW	SPS	DH	EG	APIG	APUB	FLB	WaxB	WaxP	WaxE	Pith	DM	PH	EAR L	AwnA	PPD
AUDPC	-0.219**	-0.114	-0.218**	-0.043	-0.157**	0.089	0.084	0.019	-0.064	0.137*	0.049	-0.027	-0.166**	-0.035	0.076	0.23**
TGW	1.000	-0.008	0.007	0.123*	0.111	-0.071	0.038	0.075	-0.032	-0.078	0.033	-0.007	0.235**	0.044	-0.015	0.011
SPS		1.000	0.035	-0.047	-0.043	0.047	-0.104	-0.010	0.155**	-0.158**	0.011	-0.068	0.160**	0.175	0.050	-0.287**
DH			1.000	0.002	0.016	-0.003	0.052	0.046	0.078	-0.081	-0.119	0.208**	-0.032	0.083	-0.036	-0.317**
EG				1.000	0.041	-0.147*	0.071	-0.006	-0.043	-0.066	0.000	-0.066	0.002	-0.028	0.015	-0.117*
APIG					1.000	0.036	0.047	-0.067	-0.065	0.064	-0.087	0.047	0.111	-0.067	0.058	0.109
APUB						1.000	0.029	-0.076	-0.015	0.026	0.044	-0.005	0.041	-0.052	0.090	0.006
FLA							-0.048	0.031	-0.071	0.100	-0.074	0.057	0.025	0.082	-0.047	-0.003
FLL							0.231**	0.013	0.094	-0.055	0.060	0.015	0.246**	0.116	-0.077	-0.034
FLB							1.000	0.063	-0.011	0.136*	0.043	-0.100	0.014	0.132*	0.012	0.151**
WaxS								0.484**	0.475**	0.034	0.071	0.088	0.043	-0.093	-0.100	-0.135*
WaxB								1.000	0.415**	0.065	-0.034	0.096	-0.027	-0.052	0.063	-0.36**
WaxP									1.000	-0.111	0.105	-0.031	0.008	-0.053	-0.056	-0.165**
WaxE										1.000	-0.143*	0.074	0.075	-0.113	0.050	0.124*
FOLC											-0.120*	0.044	0.100	0.068	-0.030	0.050
Pith											1.000	-0.083	-0.011	0.067	0.032	0.048
DM												1.000	-0.044	-0.059	-0.181**	-0.073
PH													1.000	-0.030	0.045	0.026
EarL														1.000	-0.095	-0.298**
AwnA															1.000	0.017*

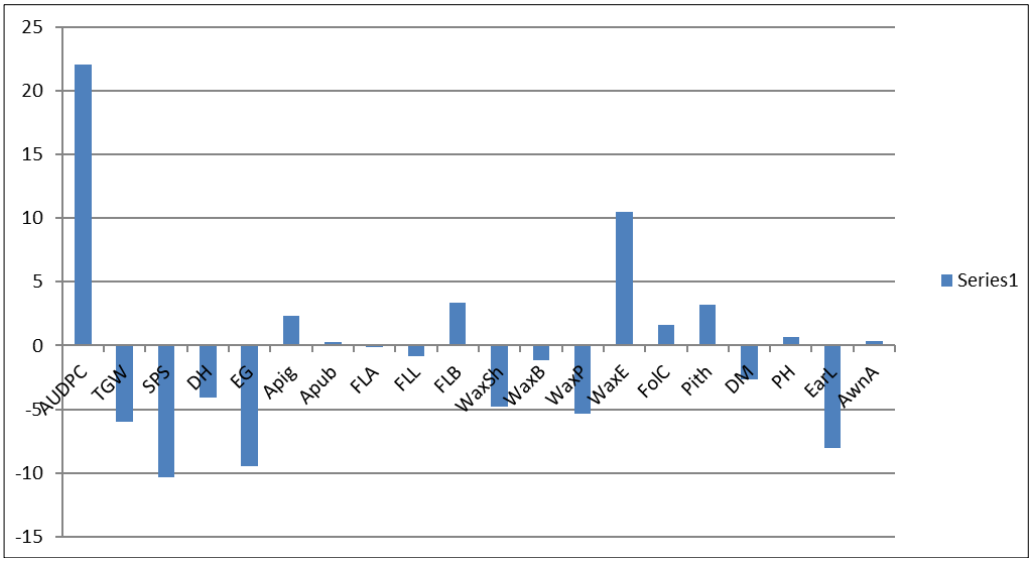

Fig 1: Histogram depicting the average value of all the traits in presence and absence of Ppd- D1

AUDPC = Area under disease progressive curve
TGW =Thousands grain weight
SPS = Seeds per 5 spike
DH =Days to heading
Wax S =Waxiness of leaf sheath
Wax B = Waxiness of blade
Wax P =Waxiness of peduncle
DM =Days to maturity
PH = Plant height
Ear L = Ear length
APIG =Auricle pigmentation
APUB =Auricle pubscence
FLA = Flag leaf attitude
FLL =Flag leaf length
FLB = Flag leaf breadth
EG =Early growth habit

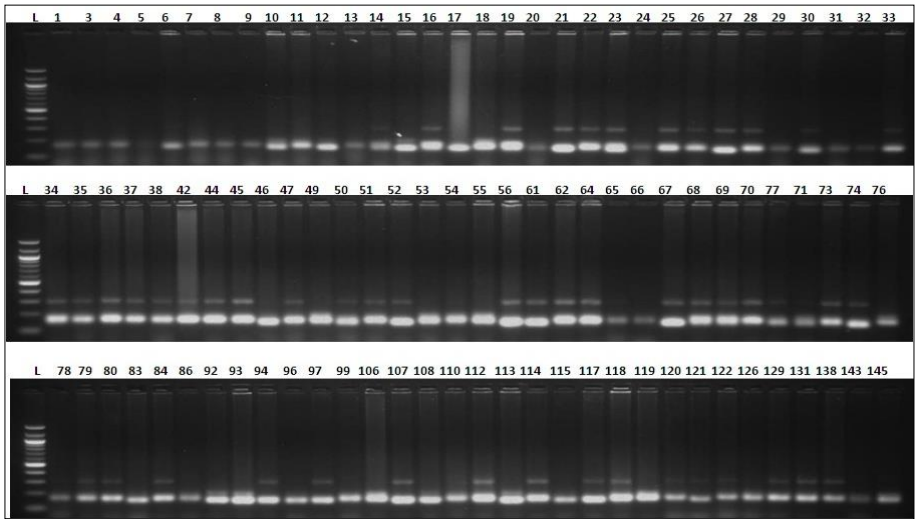

Fig 2: Validation of Ppd-D1 gene with Ppd-D1 SSR marker (288 bp)

Conclusion

All the 242 lines were genotyped with Ppd-D1 marker and observed 1:1 segregation among the population for the presence and absence of this gene.It has been reported that Ppd-D1 allele conferred a photoperiod response that might be useful for developing cultivars with closer to
optimal heading dates. Inclusion of Ppd-B1 genotypes, and more precise resolution of Ppd-D1, increased the proportion of the genotypic variance. It was observed that presence of this gene reduced 11 traits as compared with those lines which had no Ppd-D1 gene. Reduction in days to heading and plant height is the common effect observed by many researchers and days to heading also affect the spot blotch severity as reflected by area under disease in this experiment. Diversity in climate is attribute of India and variability created by Ppd-D1 gene for flowering time may be exploited in breeding programme for development of varieties for divergent regions.

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