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Full Length Research Paper

Vernalization response of winter x spring wheat derived doubled-haploids

Shivali Sharma*[12], Rajan Sharma[2] and HK Chaudhary[1]

[1]Molecular Cytogenetics and Tissue Culture Laboratory, Department of Crop Improvement, CSK HP Agricultural University, Palampur-176062, HP, India.
[2]International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru-502324, Andhra Pradesh, India.

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The effect of vernalization on growth and yield-contributing traits was studied in winter wheat (cv. Saptdhara) x spring wheat (cv. HW 3024) derived 64 doubled-haploid (DH) lines. The material was divided into two sets- with vernalization (V) treatment at 4 ± 0.5°C temperatures and without/no vernalization (NV). There were significant differences between V and NV treatments as well as between the genotypes for all the traits, including days to flowering, plant height, total tillers per plant, effective tillers per plant, spike length, spikelets per spike, and grains per spike, grain yield per plant, 1000-grain weight, and harvest index. The performance of the DH lines significantly improved under V treatment for various traits. During the early growth stages, V had a pronounced effect on growth habit of the plants with no/low injury and improved the survival rate. Two DH lines, DH 33 and DH 59 with spring type growth habit and NV requirement were identified and can be utilized for future spring wheat improvement programs.

Key words: Winter wheat, spring wheat, vernalization, doubled-haploids, agronomic traits.

INTRODUCTION

Bread wheat (*Triticum aestivum* L. em Thell) is the premier staple crop of the world and occupies a prime position among the food grain crops accounting for 216.8 million hectare area with a production of 651.4 million tonnes (FAO, 2010). Tremendous increase in wheat production was observed during green revolution with the introduction and exploitation of the 'Norin 10' dwarfing genes *Rht1* and *Rht2* for developing high-yielding semi-dwarf wheat varieties in the mid 60s. However, at present, almost a plateau has been attained in wheat production due to static acreage and continuous increase in population. For further improvement, new breeding strategies need to be identified for developing high-yielding, biotic and abiotic stress tolerant wheat varieties for cultivation under a wide range of agro-climatic conditions.

One of the breeding approaches is to utilize the winter wheat gene-pool for amelioration of spring wheat as these two groups have remained almost independent of each other due to different ecological requirements for growth and development (Akerman and Mackey, 1949). The presence of vrn_1, vrn_2, vrn_3 and vrn_4 genes (determining the vernalization requirement), and ppd_1 and ppd_2 genes (influencing photoperiodic response) have considerably altered the physiological characteristics of the winter wheat in contrast to spring wheat. Thus, winter wheat requires vernalization (V) treatment and is photoperiod sensitive, and give substantially high yield when grown under dry temperate conditions (Pugsley, 1983). The spring wheat, on the other hand, has no vernalization (NV) requirement and is photoperiod insensitive. Intercrossing the seldom crossed groups of bread wheat would bring together the two gene-pools that may increase the yield through combining complementarily components of yield (Pinthus, 1967; Grant and Mckenzie, 1970). The use of winter wheat may

*Corresponding author. E-mail: Shivali.sharma@cgiar.org. Tel: 91-040-30713570. Fax: 91-40-30713074/75.

Abbreviations: DH, Doubled-haploid; **V,** vernalization; **NV,** without/no vernalization.

contribute to spring wheat certain traits such as drought and cold tolerance (Rajaram and Skovmand, 1977), better quality, profuse tillering, large and highly fertile square spikes, good crown root system, and enhanced yield due to the incorporation of new genes (Chaudhary, 1997; Sharma and Chaudhary, 2009). Furthermore, the winter wheat may provide additional sources of resistance to stripe rust (Upadhyay and Kumar, 1975), leaf rust (Bartos et al., 1969), powdery mildew (Chaudhary and Kapoor, 1992), and *Septoria* leaf blotch (Kochumadhavan et al., 1988). Such a breeding approach has its potential utility to exploit the variability present in these two groups to evolve a wide range of superior recombinants including high-yielding, well-adapted germplasm lines, which would be suitable for cultivation under the varying ecological conditions.

Utilization of winter wheat for improvement of commonly grown spring wheat has some limitations associated with its V requirement. V is defined as the ability of a genotype to flower by a chilling treatment (Chouard, 1960). This requirement of winter wheat genotypes grown under dry temperate regions is met in the seedling stage due to extremely low temperatures (< 4°C) prevailing during winter season. Therefore, information on the phenology of winter wheat at lower latitude (less than 35°S and N), where spring wheat cultivation predominates is of great importance for their utilization in spring wheat improvement programs. Without this information, unconscious introgression of undesirable alleles into the spring wheat background may occur, resulting in the poor adaptation. Therefore, this present investigation was carried out to study the genetic differences between winter and spring wheat genotypes, and winter x spring wheat derived doubled-haploids in their response to V and NV treatments during early growth, and the effect of these differences on flowering time and yield-contributing traits.

MATERIALS AND METHODS

The experiment was carried out at the experimental farm of the Department of Plant Breeding, Himachal Pradesh Agricultural University, Palampur, India (1290 m a.m.s.l., 32°6' N, 76°3' E), representing the mid-hill zone. Based upon the growth behavior and V requirements, one elite spring wheat genotype HW 3024 having erected growth habit with NV requirement and one winter wheat genotype Saptdhara with spreading growth habit and V requirement were selected for this present study.

Generation of winter x spring wheat F_1s

In wheat, erect growth habit is dominant over spreading. Therefore, winter wheat genotype 'Saptdhara' with spreading growth habit was used as female and the erect spring wheat genotype 'HW 3024' was used as a male parent. This facilitated the identification of true F_1 plants having erect growth habit. For the success of winter x spring wheat hybridization program, the flowering periods of these two wheat ecotypes must be synchronized for a long spell. Therefore, staggered sowing of Saptdhara and HW 3024 was carried out. To fulfill the V requirement of winter wheat genotype, Saptdhara at lower latitude at Palampur having low to moderate winter temperatures, the germinating seeds were subjected to chilling treatment at 4 ± 0.5°C on wet filter paper for 30 days prior to sowing in the field. The F_1 seeds were generated by crossing Saptdhara with HW 3024.

Production of doubled-haploid lines

The F_1s of the cross between Saptdhara x HW 3024 were pollinated with maize pollen and 64 doubled-haploid lines were developed following wheat x maize system (Laurie and Bennett, 1988). A standardized protocol was used to produce haploid plantlets and doubled-haploids (DH) following colchicine treatment (Chaudhary et al., 2002; Sharma et al., 2004).

Vernalization and no vernalization treatment

The seeds of parental genotypes, Saptdhara and HW 3024 and 64 DH lines were divided into two sets: one for vernalization (V) and another for without/no vernalization (NV) treatments. In V set, the germinating seeds of the parental genotypes and DH lines were given V treatment at a temperature of 4 ± 0.5°C for 30 days prior to sowing in the field, whereas the seedlings in another set kept for NV treatment were established at room temperature one week prior to the end of treatment in the V set so as to transplant material from both the treatments at the same time and at approximately same growth stage. The seedlings were transplanted in the field. The experiment was laid-out in a split-plot design with three replications. The V and NV treatments were assigned to the main plots and the genotypes to the sub-plots. The plot size was 1.0 m single row with inter-row and inter-plant spacing of 23 cm and 5 to 6 cm, respectively. The observations were recorded on plot basis for field survival of the plants (%), growth rate (injury to the plant, if any), growth behavior (spreading, semi-spreading, and erect) during the initial vegetative stage, and days to 50% flowering, and on five randomly selected plants for plant height, total tillers per plant, effective tillers per plant, spike length, spikelets per spike, grains per spike, grain yield per plant, 1000-grain weight and harvest index (%). Field survival as a measure of winter hardiness was calculated as the percentage of plants survived one month after transplanting. The growth behavior of the plants was observed one month after transplanting up to heading stage at three days interval. Growth rate/injury to the plants at tillering stage under both the conditions was visually estimated on a 0 to 9 scale (0: no damage; 1: slightly yellowed leaf tips; 2: half yellowed basal leaves; 3: fully yellowed basal leaves; 4: whole plant slightly yellowed; 5: whole plant yellowed and some plants withered; 6: whole plant yellowed and 10% plant mortality; 7: whole plant yellowed and 20% plant mortality; 8: whole plant yellowed and 50% plant mortality; 9: all plants killed). The tillers with sterile spikelets were also recorded.

The data were analyzed as a split plot design with treatments (V vs. NV) as main plots and genotypes as sub-plots using GenStat 12.1. Mean, range, and variance were calculated for each trait in each treatment as well as for each trait in three groups separately. The means were tested using Newman-Keuls test (Newman, 1939; Keuls, 1952) and variances using Levene (1960) test.

RESULTS

The temperature and rainfall regimes during the cropping season (*rabi* 2003-04) at Palampur is given in Figure 1. At the time of germination and seedling stage, the

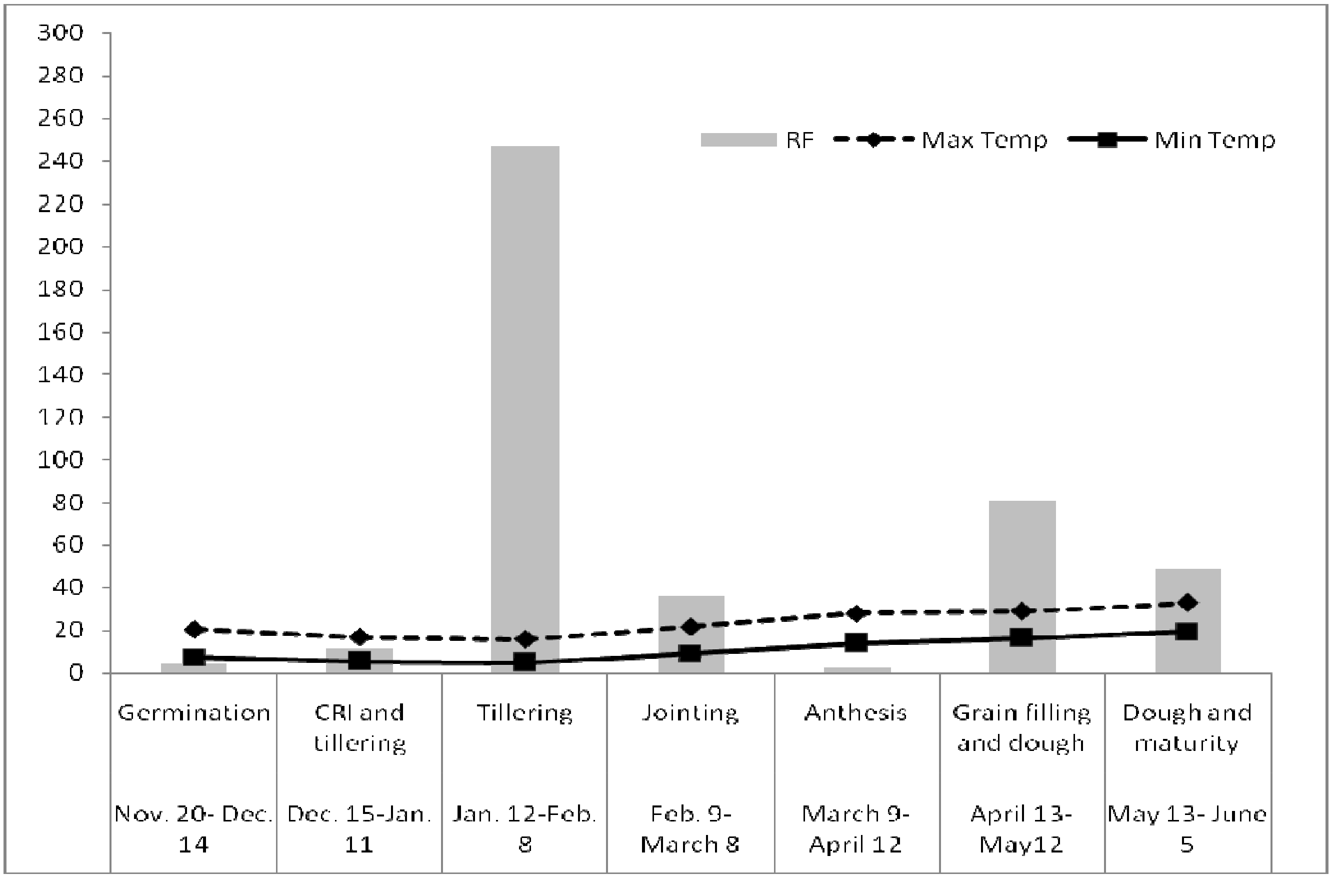

Figure 1. Temperature regimes and rainfall pattern during the cropping season (*rabi* 2003-04).

temperature under field conditions was above the critical limit (0 to 4°C) for V requirement of winter wheat genotype, Saptdhara and the winter type DH lines. The analysis of variance showed significant differences between V and NV treatments as well as between the genotypes for all the traits, viz., days to flowering, plant height, total tillers per plant, effective tillers per plant, spike length, spikelets per spike, grains per spike, grain yield per plant, 1000-grain weight and harvest index (Table 1).

Field survival of the plants after the fall of winter varied from 68.3 to 100% under NV. Only four DHs (DH 18, DH 40, DH 52 and DH 71) had 100% field survival under NV and showed erect growth behavior. None of the lines with spreading/semi-spreading growth behavior had 100% survival under NV. Field survival of the plants improved significantly under V, and varied from 88.3 to 100.0%. About 80% DH lines had 100% field survival (Figure 2), majority of which were spreading type (53%), 16% semi-spreading, and 31% erect in growth behavior. The growth of the plants was comparatively poor under NV and over 50% of the DH lines suffered high injury (≥ 5 rating on a scale of 0-7) due to low temperature. Growth rate was significantly improved under V and about 33% DH lines had no damage (0 rating), 52% lines had slightly yellowed leaf tips (1 rating) and only 16% DH lines had half yellowed basal leaves (2 rating).

The performance of DH and parental lines for various traits under NV and V conditions could compare the effect of V on early growth and yield attributes in wheat genotypes. Based on per se performance, under NV condition, 59% DH lines had spreading growth habit, whereas after V treatment, only 52% DH lines were spreading type (Table 2). Five DH lines, DH 4, DH 44, DH 58, DH 16, and DH 17 with spreading growth habit under NV showed differential growth behavior under V. Of these, three DH lines, DH 4, DH 44, and DH 58 had semi-spreading growth habit while the remaining two lines (DH 16, and DH 17) had erect growth habit under V. Eight DH lines exhibited semi-spreading growth habit under both the conditions whereas six DH lines DH 3, DH 35, DH 46, DH 55, DH 60 and DH 64 showed differential growth habit under two conditions. These six lines were semi-spreading under NV but erect under V. About 19% DH lines were erect type under both the conditions (Table 2). As stated earlier, injury to the plants was reduced under V in comparison to NV conditions.

The mean values of parents and DH lines for

Table 1. Analysis of variance (split plot design) for different traits studied.

Source	df	Day to flowering	Plant height (cm)	Total tiller	Effective tiller	Spike length (cm)	Spikelet/ spike	Grain/ spike	Grain yield/plant (g)	1000-grain weight (g)	Harvest index (%)
Replication	2	1.52	17.76	0.50	0.37	0.90	1.78	11.26	0.39	1.63	79.10
A	1	7161.83*	21620.48*	52.95*	215.91*	99.78*	172.01*	29510.85*	914.53*	3043.40*	4590.51*
Error a	2	7.69	13.03	0.10	0.10	0.38	2.04	3.39	0.20	0.88	57.67
B	65	797.77*	797.03*	1.45*	1.65*	2.60*	9.68*	288.94*	4.08*	126.13*	263.33*
AB	65	30.50*	122.48*	1.29*	1.20*	2.81*	11.02*	321.30*	4.50*	106.69*	212.86*
Error b	260	3.57	5.46	0.25	0.23	0.22	1.16	4.79	0.16	0.77	24.97
CD (A)		1.20	1.56	0.14	0.14	0.27	0.62	0.80	0.19	0.41	3.28
CD (B)		2.17	2.68	0.58	0.54	0.53	1.24	2.51	0.46	1.01	5.73
CD (AB)		3.06	3.79	0.82	0.77	0.75	1.75	3.55	0.64	1.42	8.10
Mean (V)		117.1	82.3	3.8	3.8	10.8	19.4	44.9	5.3	42.5	37.3
Mean (NV)		125.6	67.5	3.1	2.3	9.8	18.1	27.2	2.2	36.9	30.1

*Significant at P ≤ 0.05. A: Main plot factor- Treatments: Vernalization (V) and without/no vernalization (NV), B: Sub plot factor- genotypes, AB: Interaction.

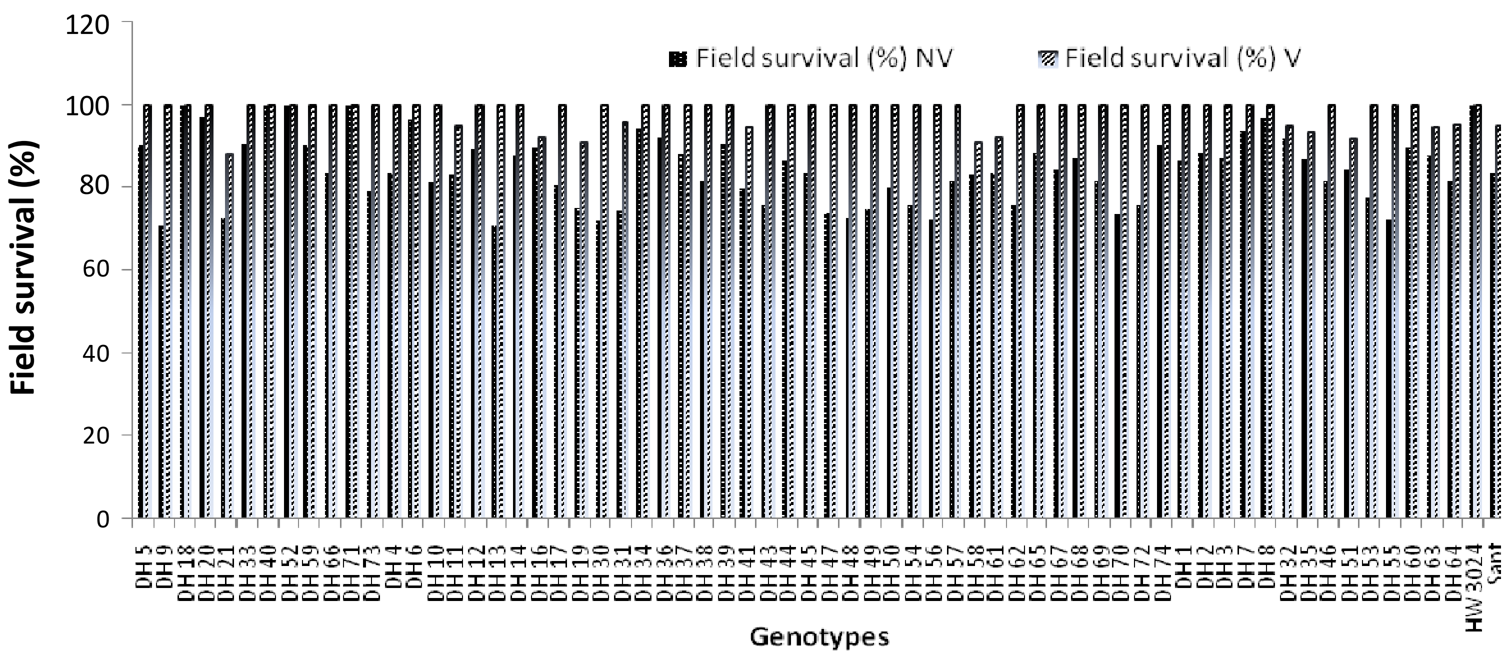

Figure 2. Field survival of the genotypes (DH lines and parents) under without/no vernalization (NV) and vernalization (V) treatments.

Table 2. Growth behavior and vernalization requirement of the doubled-haploid (DH) lines and the parental genotypes.

S/N	DH line	Growth behavior		Day to 50% flowering		S/N	DH line	Growth behavior		Day to 50% flowering	
		NV	V	NV	V			NV	V	NV	V
	Spreading: High vernalization requirement						**Semi-spreading: Moderate vernalization requirement**				
1	DH 72	S	S	149.7	136.3	22	DH 62	S	S	123.3	114.7
2	DH 39	S	S	147.7	137.3	23	DH 6	S	S	120.3	114.3
3	DH 45	S	S	147.7	144.7	24	DH 70	S	S	120.3	107.0
4	DH 38	S	S	146.3	136.3	25	DH 17	S	E	118.0	105.3
5	DH 31	S	S	146.0	135.7	26	DH 36	S	S	118.0	114.0
6	DH 65	S	S	145.7	134.0	27	DH 58	S	SS	116.7	109.3
7	DH 14	S	S	144.0	128.0	28	DH 51	SS	SS	138.3	130.3
8	DH 10	S	S	143.0	133.7	29	DH 1	SS	SS	136.3	128.7
9	DH 50	S	S	141.7	131.3	30	DH 53	SS	SS	125.0	121.3
10	DH 49	S	S	141.3	132.7	31	DH 46	SS	E	121.3	112.3
11	DH 13	S	S	141.0	132.0	32	DH 7	SS	SS	121.0	115.3
12	Saptdhara	S	S	144.7	136.0	33	DH 63	SS	SS	118.0	110.3
	Semi-spreading: Moderate vernalization requirement					34	DH 55	SS	E	117.7	115.7
1	DH 47	S	S	137.7	120.7	35	DH 64	SS	E	117.7	113.7
2	DH 61	S	S	137.0	127.7	36	DH 2	SS	SS	117.3	105.7
3	DH 48	S	S	135.7	126.3	37	DH 32	SS	SS	117.0	121.0
4	DH 11	S	S	135.3	130.0	38	DH 35	SS	E	116.3	114.3
5	DH 67	S	S	134.3	121.0	39	DH 8	SS	SS	115.7	105.0
6	DH 44	S	SS	131.3	116.3	40	DH 3	SS	E	114.3	103.3
7	DH 30	S	S	130.7	116.0	41	DH 60	SS	E	110.0	107.0
8	DH 68	S	S	130.3	116.7		**Erect: No/low vernalization requirement**				
9	DH 69	S	S	130.0	120.3	1	DH 20	E	E	115.7	110.3
10	DH 43	S	S	129.7	114.3	2	DH 66	E	E	115.7	110.0
11	DH 41	S	S	129.0	113.7	3	DH 21	E	E	114.3	106.3
12	DH 57	S	S	128.3	120.3	4	DH 5	E	E	113.3	109.7
13	DH 37	S	S	127.0	114.0	5	DH 9	E	E	113.3	104.3
14	DH 12	S	S	126.7	119.3	6	DH 73	E	E	113.3	110.3
15	DH 74	S	S	126.7	112.3	7	DH 18	E	E	113.0	107.0
16	DH 34	S	S	126.3	109.7	8	DH 71	E	E	109.7	104.7
17	DH 4	S	SS	126.0	116.7	9	DH 59	E	E	108.3	105.7
18	DH 16	S	E	125.7	115.7	10	DH 52	E	E	103.7	102.0
19	DH 56	S	S	125.3	111.0	11	DH 33	E	E	103.3	101.3
20	DH 19	S	S	124.3	118.3	12	DH 40	E	E	102.0	100.3
21	DH 54	S	S	123.7	111.3	13	HW 3024	E	E	104.3	100.7

NV = Without/no vernalization treatment, V = Vernalization treatment.

agronomic traits were significantly increased under V. Day to 50% flowering were significantly reduced under V in comparison to NV. The 50% flowering varied from 102.0 to 149.7 days under NV and 100.3 to 144.7 days under V conditions (Table 3). The genotypes flowered significantly earlier upon V although they showed a varied response to V. The number of days to 50% flowering was reduced by 17 days in DH

Table 3. Range, mean and variance for different traits under without/no vernalization (NV) and vernalization (V) treatments

Character	Treatment	Range	Mean*	Variance**	F value	Probability
Field survival (%)	NV V	70.7 - 100.0 88.3 - 100.0	84.5 ± 1.01[B] 98.6 ± 0.36[A]	67.83 8.78	38.61	<0.0001
Days to flowering	NV V	102.0 - 149.7 100.3 - 144.7	125.6 ± 1.54[A] 117.1 ± 1.35[B]	156.46 119.63	1.85	0.176
Plant height (cm)	NV V	35.5 - 95.6 50.4 - 104.9	67.5 ± 1.58[B] 82.3 ± 1.47[A]	164.55 141.95	0.42	0.518
Total tillers / plant	NV V	1.5 - 4.3 2.6 - 6.0	3.1 ± 0.07[B] 3.8 ± 0.09[A]	0.33 0.58	4.26	0.041
Effective tillers / plant	NV V	0.6 - 3.3 2.6 - 6.0	2.3 ± 0.07[B] 3.8 ± 0.09[A]	0.37 0.58	2.87	0.093
Spike length (cm)	NV V	8.1 - 12.1 9.0 - 12.6	9.8 ± 0.12[B] 10.8 ± 0.11[A]	1.03 0.77	1.60	0.208
Spikelets / spike	NV V	13.9 - 23.0 16.4 - 22.5	18.1 ± 0.26[B] 19.4 ± 0.19[A]	4.49 2.41	7.11	0.009
Grains / spike	NV V	2.3 - 44.6 23.5 - 68.5	27.2 ± 1.36[B] 44.9 ± 1.08[A]	121.77 76.90	3.59	0.060
Grain yield / plant (g)	NV V	0.1 - 4.2 2.6 - 8.2	2.2 ± 0.11[B] 5.3 ± 0.17[A]	0.85 1.91	11.88	0.001
1000-grain weight (g)	NV V	18.3 - 51.1 32.7 - 57.5	36.9 ± 0.87[B] 42.5 ± 0.64[A]	50.39 27.21	4.75	0.031
Harvest index (%)	NV V	2.3 - 45.8 19.0 - 49.9	30.1 ± 1.30[B] 37.3 ± 0.84[A]	111.43 46.77	8.08	0.005

*Differences between means of NV and V treatments were tested by Newman-Keuls test. Mean followed by same letter indicate non-significant and different letter indicate significant differences at P = 0.05.** Variance homogeneity tested by Levene's test

34 and DH 47 after V, whereas DH 33, DH 35, DH 40, DH 52, and DH 55 flowered two days earlier under V in comparison to NV. Although all the traits were significantly improved under V, grain yield per plant was improved to the greatest extent after V.

Maximum improvement in this trait under V may be attributed mainly to the increase in number of effective tillers per plant as well as grains per spike after V. Harvest index, total tillers per plant, plant height, field survival, 1000-grain weight, spike length, and spikelets per spike also showed significant increase after V treatment in comparison to NV (Table 3).

Effect of V was further studied in relation to growth habit of the plants. True winter types have high V requirement followed by facultative types having moderate V requirement and spring types with no/low V requirement. In this present study, the V requirement of the plant was predicted from number of days to 50% flowering under NV conditions (Table 2). The winter wheat parent 'Saptdhara' and the spring wheat parent 'HW 3024' showed spreading and erect growth habits, respectively under both the environments after germination. Under NV, an unambiguous classification of the DH lines from the cross between Saptdhara x HW 3024 with respect to growth habit and V requirement was obtained. About 59% DH lines showed spreading, 22% semi-spreading and 19% erect growth habit based on per se growth habit. Lines requiring high V remained in the vegetative stage until late in the growing season and took more number of days to 50% flowering. In general, plants with spreading growth habit took more number of days to flowering (average 133 days; range 116.7 to 149.7 days) in comparison to plants with erect growth habit (average 110 days; range 102 to 115.7 days), whereas DH lines with semi-spreading growth habit had an overlapping range (average 120; range 110.0 to 138.3 days). DH lines with spreading growth habit showed two types of behavior for V requirement in terms of number of days to 50% flowering. In one group, 11 DH lines (DHs 75, 39, 45, 38, 31, 65, 14, 10, 50, 49, and 13) performed almost similar to true winter wheat parent Saptdhara (145 days) and took about 141 to 150 days (144.9 days) to 50% flowering. The remaining 27 lines flowered earlier than Saptdhara, but later than HW 3024 and performed

Table 4. Agronomic performance of three groups under without/no vernalization (NV) and vernalization (V) treatments.

Character	Treatment	Erect	Semi-spreading	Spreading	Spring wheat parent	Winter wheat parent
No. of DH lines		12	41	11	HW 3024	Saptdhara
Field survival (%)	NV	89.5 ± 3.11^{B}	83.7 ± 1.1^{B}	80.9 ± 1.95^{B}	100	83.3
	V	99 ± 0.98^{A}	98.2 ± 0.49^{A}	99.6±0.37^{A}	100	95
Days to flowering	NV	110.5 ± 1.44^{A}	125.1 ± 1.13^{A}	144.9±0.88^{A}	104.3	144.7
	V	106 ± 1.04^{B}	115.5 ± 1.05^{B}	134.7 ± 1.29^{B}	100.7	136
Plant height (cm)	NV	76.1 ± 3.79^{B}	68.7 ± 1.56^{B}	55.9 ± 4.1^{B}	63	45.4
	V	89.6 ± 2.69^{A}	83.1 ± 1.74^{A}	73.5 ± 3.53^{A}	76.3	62.4
Total tillers / plant	NV	3.1 ± 0.11^{B}	3.1 ± 0.09^{B}	2.9 ± 0.21^{B}	2.4	2.7
	V	3.9 ± 0.23^{A}	3.8 ± 0.12^{A}	3.8 ± 0.2^{A}	2.9	3.2
Effective tillers / plant	NV	2.4 ± 0.1^{B}	2.4 ± 0.09^{B}	1.9 ± 0.19^{B}	2.4	0.6
	V	3.9 ± 0.23^{A}	3.8 ± 0.12^{A}	3.8 ± 0.2^{A}	2.9	3.2
Spike length (cm)	NV	10.5 ± 0.33^{A}	9.8 ± 0.14^{B}	9.1 ± 0.21^{B}	8.4	8.3
	V	11 ± 0.27^{A}	10.7 ± 0.14^{A}	11 ± 0.19^{A}	9.1	10.6
Spikelets / spike	NV	18.3 ± 0.6^{A}	18.3 ± 0.33^{B}	17.9 ± 0.6^{B}	13.9	15.1
	V	18.6 ± 0.48^{A}	19.4 ± 0.21^{A}	20.7 ± 0.39^{A}	17	19.7
Grains / spike	NV	32.9 ± 1.58^{B}	28.3 ± 1.66^{B}	18.2 ± 3.11^{B}	35	2.3
	V	45.9 ± 2.73^{A}	46.3 ± 1.24^{A}	37.8 ± 2.64^{A}	43.4	54.5
Grain yield / plant (g)	NV	2.6 ± 0.13^{B}	2.3 ± 0.14^{B}	1.5 ± 0.3^{B}	2.4	0.2
	V	5.5 ± 0.41^{A}	5.4 ± 0.2^{A}	4.8 ± 0.52^{A}	3.8	4.9
1000-grain weight (g)	NV	40.7 ± 1.15^{A}	36.9 ± 1.01^{B}	33.5 ± 3.14^{B}	34.1	31.8
	V	41.9 ± 1.05^{A}	42 ± 0.78^{A}	45.5 ± 2.05^{A}	35.7	44
Harvest index (%)	NV	35.2 ± 1.87^{A}	30.9 ± 1.44^{B}	22.1 ± 3.86^{A}	44.7	8.5
	V	38.7 ± 1.97^{A}	38.3 ± 0.9^{A}	30.7 ± 2.07^{A}	41.4	50.5

*Differences between means of NV and V treatments were tested by Newman-Keuls test. Mean followed by same letter indicate non-significant and different letter indicate significant differences at P = 0.05.

similar to the lines with semi-spreading growth habit (average 127.3 days; range 116.7 to 137.7 days). Based on this information, 64 DH lines and the parental genotypes were divided into three groups. The winter wheat parent Saptdhara and 11 DH lines with spreading growth habit having high V requirement were placed in spreading type (true winter) group, whereas 41 DH lines with spreading (27) and semi-spreading (14) growth habit had moderate V requirement and were placed into second group, semi-spreading (facultative winter) type. The remaining 12 DH lines and spring wheat parent HW 3024 with erect growth habit had no/low V requirement to enter into the reproductive phase and were placed into erect (spring) type group (Table 2).

The effect of V was the highest in genotypes with spreading growth habit followed by semi-spreading and erect (Table 4). Of the various traits, grain yield per plant of the DH lines belonging to spreading group was improved to the maximum extent (about 220% increase over NV) followed by grains per spike (about 108% increase over NV) and effective tillers per plant (about 100% increase over NV). The number of days to flowering was reduced significantly under V among all the three groups; however, the reduction was comparatively high with the genotypes having spreading and semi-spreading growth habit (Table 4).

DISCUSSION

Winter wheat requires V to enter into reproductive phase. For the success of winter x spring wheat hybridization in spring wheat improvement, it is imperative to study the V requirement and its effect on growth of recombinants and genotypes. The completely homozygous DH lines generated from winter x spring wheat F_1s is an efficient material for such studies. This present study revealed that the V treatment given at seedling stage affected different stages of plant growth. During initial growth stages, V affected growth behavior and growth rate of plants, whereas at the later growth stages, V treatment had significant and positive effect on various agronomic traits such as plant height, days to 50% flowering, total tillers per plant, effective tillers per plant, spike length, spikelets per spike, grains per spike, grain yield per plant, 1000-grain weight, and harvest index.

The performance of the DH lines improved under V for various traits. During the early growth stages, the V had a pronounced effect on growth habit of the plant with no/low injury to the plants and improved the survival rate under low latitude where low temperature prevails during the crop growth. Better survival as well as better growth of plants after V may be attributed to their improved winter hardiness suggesting that V and winter hardiness ability of a genotype may be governed by the same or closely linked genes. Among the three groups, field survival of the plants with spreading growth habit improved to a greater extent after V indicating that cold tolerance in winter wheat is linked to V requirements. Doll et al. (1989) observed a positive effect of V on winter hardiness in barley. The plants requiring V remained in the vegetative stage until late in the growing season under NV conditions and produced a few tillers, which were mostly sterile. After V, the trigger to induce reproductive phase was activated and the plants entered into the reproductive phase comparatively early. The performance of a line for other traits such as spike length, spikelets per spike, grains per spike, grain yield per plant and grain size (1000-grain weight) also improved after V, which in turn led to enhanced yield. Fowler et al. (1996) while studying the relationship between low-temperature tolerance and V response in wheat and rye found that expression of low-temperature tolerance genes is dependent on the stage of V. We also observed increased low temperature tolerance with V. Based on days to flowering, in general, DH lines with spreading growth habit had moderate to high V requirement, whereas lines with erect growth behavior had no/low V requirement. Roberts (1990) observed a close linkage between a gene controlling prostrate growth habit with *Fr*1 and *Vrn*1 on chromosome 5A. Hence, it can be inferred that growth habit of the plant under field conditions can serve as an indicator for V requirement. However, it is not possible to measure the intensity of V required solely based on growth habit.

Identification of promising spring type DH lines

The DH lines derived from a winter x spring wheat F_1 will represent different recombinants. Success of such breeding approach for spring wheat improvement will depend upon the identification of promising spring type DH lines with desirable traits from winter wheat. Therefore, in this present study, the performance of DH lines was compared with spring wheat parent HW 3024 under NV condition to identify promising spring type DH lines. The NV treatment in this present study represents the natural environmental conditions prevailing at low latitude areas like Palampur, where spring wheat cultivation predominates. Therefore, the lines performing better than or at par with the spring wheat parent HW 3024 for various traits under NV condition will be suitable for spring wheat improvement programs in low latitude areas. Only four DH (DHs 59, 52, 33, and 40) flowered at par (102-108 days) with HW 3024 (104 days), of which only two DHs (DHs 33 and 59) were significantly high yielding (3.4 and 3.1 g grain yield per plant, respectively) and remaining two performed at par with HW 3024 (2.4 g). These lines except DH 52 also performed better for other traits such as total tillers, effective tillers, spike length, spikelets per spike, grains per spike, grain yield per plant and grain weight. Twenty-two DH lines had significantly high effective tillers per plant (2.7 to 3.3) compared to HW 3024 (2.4), of which 14 DHs also yielded significantly high grain yield per plant. However, these DHs, except DH 33 showed delayed flowering (113 to 133 days). Similarly, 51 DH lines had significantly high spike length (8.9 to 12.1 cm). However, except DHs 52, 59, and 33, all were late flowering (110 to 150 days). About 30% DHs yielded significantly low grain yield per plant (0.3 to 2.0 g) that was mainly due to low grains per spike and high number of sterile tillers in these lines. The result shows that these DH lines are the recombinant between winter and spring wheat genotype of which only three recombinants (DHs 52, 59, and 33) are desirable and remaining exhibited linkage drag. Ten DH lines had significantly high grains per spike (39.0 to 44.6) compared to HW 3024 (35.0); 19 had significantly high grain yield per plant (2.8 to 4.2 g) compared to HW 3024 (2.4 g); and 3 had significantly high 1000-grain weight (37.3 to 41.3 g) compared to HW 3024 (34.1 g). However, majority of the identified promising DH lines for different traits took comparatively more number of days for flowering, which could be attributed to the introgression of genes from winter wheat parent resulting in delayed flowering.

It can be concluded that effective utilization of winter wheat genotypes for amelioration of spring wheat genotypes depends on identification of recombinants with no V requirement. Identification of these recombinants should not be based on growth behavior, but on overall performance of a plant for various agronomic traits. In this present study, we could identify two high yielding DH lines DH 33 and DH 59, which do not require V and are superior to spring wheat parent HW 3024. These DHs being fixed lines can be used as parents in spring wheat improvement program as well as can also be recommended for cultivation after multi-location and multi-year evaluation.

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