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Growth Habit and Vernalization Requirement in some of Iranian Bread Wheat Cultivars

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ABSTRACT: To determine the action of vernalization and growth habit variation in cultivars of bread wheat (*Triticum aestivum* L.), this research was carried out in greenhouse of Agricultural and Natural Resources Research Center of South Khorasan in 2013-2014 growing seasons. The treatments consisted of 40 cultivars of wheat and 5 levels of vernalization periods (0, 15, 30, 45 and 60 days) at ambient temperature in January and conducted with randomized complete block design with two replications. Vernalization fulfillment was determined using the final leaf number method. Fitting formula was used for each genotype. Then α and β obtained from this fitting used genotypes grouped according to the severity of its response to vernalization was used (using Wards method for cluster analysis with SPSS software). The results showed that Soissons, Zare, C.86.5, Azar2, Gaspared and Gaskogen have a highest leaves with 11 leaves in no-vernalized conditions and divided into separate groups. Roshan, Pishgam, Mihan, Tabasi, Alvand, Dena, Bezostaya, and Karaj3 were intermediate in leaf product. The rest of these were in the lowest group.

Key words: Consistency, Final leaf number, Growth habits, Vernalization requirement, Vernalization fulfillment, Wheat.

INTRODUCTION

Transition from vegetative to reproductive phase in wheat requires exposure to a cold period, referred as vernalization requirement. The process of vernalization occurs in buds, hence soaked seeds, seedlings, immature seeds forming in mother plant, and even calluses derived from fetal tissue cultures respond to vernalization (Gardner and Barnett, 1990; Whelan and Schaalje, 1992; Cao and Moss, 1991). Factors influencing vernalization include age, cold (temperature) intensity and duration, chilling method, genotype, development phase and growth hormones (Rawson *et al.,* 1998; Ortiz-Ferrara *et al.,* 1998). Effective temperature ranges to meet wheat vernalization requirement are between zero and 18°C and temperatures higher than 30°C can adversely affect it (Slafer and Whitechurch, 2001; Rosenzweig and Tubiello, 1996). Flowering time in wheat can be described as a function of (1) the number of leaves produced by the main-stem; (2) the rate of leaf appearance (i.e. the inverse of phyllochron); (3) the thermal time between flag leaf appearance and flowering; and is under the control of temperature and photoperiod (McMaster, 2005).

He *et al.,* (2012) analyzed the correlations between the varietal parameters and determined the minimum number of parameters that need to be estimated in order to accurately simulate the effect of the genotype and environment on wheat anthesis date. They concluded that variation in both final leaf number and anthesis date in response to the environment and the genotype was predicted with a mean error of 0.55 leaves and 3.94 d, respectively. Also, the phyllochron was reasonably well estimated and showed a positive association with earliness per se (Spearman's coefficient of rank correlation = 0.59)

Slafer and Whitechurch (2001) reviewed the results of published research and stated that vernalization takes place at the range of -1 to 1°C to 13-18°C, depending on genotype and other conditions of vernalization (except temperature); but the most effective temperature range for vernalization requirement is 4.1-10.6°C.

Vernalization requirement is defined as the minimum number of days after which the plant becomes insensitive to vernalization (Wang *et al.,* 1995). Classification of varieties as spring and winter according to vernalization has been used from the past and no classification more reliable than winter, facultative, and spring was provided yet. Gardner and Barnett (1990) studied vernalization responses of wheat cultivars and divided them into three types of qualitative (6-8 weeks at 6°C), quantitative (2-4 weeks at 2°C), and neutral; this classification is consistent with the results of Rawson *et al*. (1998). But according to Wang et al., (1995) the classification relies further on cultivation date consistent with a cultivar, rather than the nature of vernalization response of that cultivar, so some spring varieties may need vernalization, or in contrary, winter varieties may require an extended vernalization. Some cultivars extensively grown throughout the world do not basically require vernalization or need a little (Wang et al., 1995). Based on the reducing effect of vernalization on the final number of leaves, and the relationship between plant age and duration of vernalization in a wide range of wheat, Wang et al., fitted some equations and believed that vernalization can be quantified based on the reaction of a genotype (the coefficients ? and ? in the mentioned equations). According to them, among methods used to determine biological and phenological stages of cereals, only the final leaf number can clearly demonstrate essential biological changes. It is also a good approach which directly reflects phenological changes such as transition from vegetative to reproductive phase and a good morphological index for determining vernalization fulfillment (Fowler et al., 1996).

Mahfoozi and Sassani (2008) studied vernalization requirements of 8 wheat cultivars in field and controlled conditions using the final leaf number, and determined that vernalization requirement of Noorstar, Azar 2, and Sardari were about 11, 8, and 8 weeks, respectively, in 2002. The figures changed to 12, 8, and 7 weeks in 2003. Sharifi *et al.* (2011) studied 29 cultivars and lines of wheat at 9 vernalization levels in growth chambers based on days to heading. The estimated vernalization requirement for cultivars Shahriar, Gaskogen, C-80-4, C-80-6, Soisons, MV-17, and C-81-14 were 2, 3, 3, 3, 4, 4, 4, and 4 weeks, respectively, at 3 °C.

Gardner and Barnett (1990) calculated vernalization of wheat varieties in terms of a quantity called chilling unit and declared that the amount of chilling unit required to fulfill vernalization requirement is natural in the field. According to these researchers, high fluctuation in temperatures in the field can increase the effect of coldness on fulfillment of vernalization requirement of wheat; this occurs due to an increase in chilling units for fulfilling vernalization requirement in the refrigerator (compared to field).

Wheat responds to vernalization requirement through reducing the days until flowering; during which it is affected by vernalization (Vrn), photoperiod, and earliness per se genes. Dominancy of vernalization gene (Vrn-A1) accelerates initiation of leaves, while Vrn-A2 decelerates initiation of leaves and flowers. Some studies have shown that although vernalization and photoperiod are controlled by two separate genes, vernalization interacts with photoperiod, and the effect of low temperatures on wheat vernalization is lower in short days than long days (Ortiz-Ferrara *et al.,* 1998). Vernalization requirement of wheat is also reduced by aging (Wang *et al.,* 1995; Whelan and Schaalje, 1992). This study was conducted to determine the response of bread wheat lines and cultivars to vernalization and its range, as well as its effects on the final number of leaves and day to heading. The findings can be applied in wheat breeding programs.

MATERIALS AND METHODS

This research was performed in the intelligent research greenhouse of the South Khorasan Agricultural and Natural Resources Research Center located at 32°52'N and 59°13'E, with an altitude of 1280 meters above sea level in growing season 2013-2014.

The experiment included treatments of vernalization (at 5 levels of zero, 15, 30, 45, and 60 days) and 40 lines and cultivars of wheat (Bezostaya, Alvand, Ghods, Shahriar, Sepahan, Arta, Parsi, Falat, Moghan 3, Arg, Kavir, Maroon, Roshan, Soisons, WS-82-9, Gaskogen, Azar 2, Gaspard, Darya, Zagros, Tabasi, Mahooti, Mihan, Zare, Dena, Sorkhtokhm, Ofogh, Nicknejad, Dez, Pishgam, Karaj 3, Rasool, Chamran, Pishtaz, Atrak, Darab 2, Star, C-85-D-13, Morvarid, and C-86-5). They were studied in a completely randomized design with two replications.

Disinfected seeds were planted in 300 cm^3 pots (6 seeds per pot). The pots were kept at 20°C for 24 h before planting and watering aiming at beginning the germination process. They were then transferred to open space and field conditions and were completely buried in pits already prepared. Day length and light intensity were applied normally.

To induce vernalization, the replicates of each treatment were transferred to the intelligent greenhouse at every 15 days. The first treatment (no-vernalization) was planted directly in the greenhouse. Thinning operations of the pots were performed after germinating in field conditions and seedlings were reduced to four per pot. In addition, those seedlings with almost similar growth conditions were selected to minimize its interfering effect with the vernalization treatments. Greenhouse temperature was maintained at 20±2°C, always above 18°C, throughout the experiment. Also to induce flowering, lighting hours and light intensity were adjusted to 16 hours and 400 $\mu mol/m^2 \cdot s$ per day. Thus, vernalization was not occurred in the greenhouse and no interference existed with the vernalization treatments. However, in order to distinguish different durations of vernalization treatments from the interval between sowing to heading, the time from transition to the greenhouse until complete emergence of wheatear (exit of the last spikelet from the leaf sheath in the main stem) was used for analysis of the effects of vernalization treatments on vegetative period length (days to heading). Wheatear emergence date of each replication was recorded on a daily basis over a period of near 3 months after transfer to the greenhouse. The final number of leaves in the main stem was counted in 3 plants of each replication from germination until heading once every ten days. Of course this was only done on cultivars flowered in the greenhouse conditions, and the final number of leaves of those flowered beyond this range was not recorded.

The method of Wang *et al.* (1995) was used to group the studied cultivars and lines in terms of response to vernalization. The following equations have been established in this method, relying on the effect of vernalization on reducing the final number of leaves and the relationship between plant age and vernalization duration in a wide range of wheat:

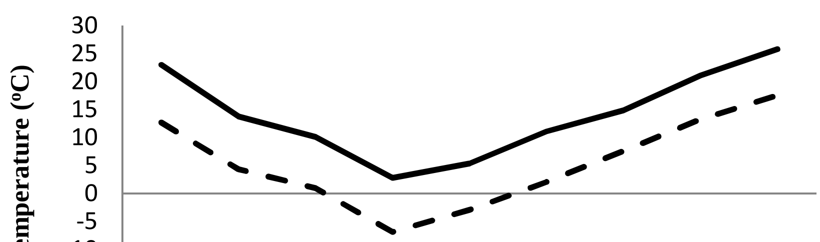
$$F_0 - 6 = L_i + \beta T_v \qquad F_i - 6 = \alpha - \beta T_v$$

Where F_0 is the final number of leaves on main stem in the absence of vernalization; F_i the final number of stem leaves in a vernalization treatment; L_i leaf stage at the beginning of insensitivity to vernalization; and T_v the number of vernalization days. α and β are the equation's coefficients and have a physiological concept; α represents the variable number of leaves, i.e. α indicates that how many leaves can potentially be reduced as a result of vernalization. It is biologically equivalent to the number of leaves of no vernalization plants at the beginning of no vernalization insensitivity (Slafer and Whitechurch, 2001). According to this study, in no vernalization plants, FLN = 6 is approximately equal to α, and β is the exchange rate between leaf number and duration of vernalization. Thus, the intensity of a genotype response to vernalization can be quantified based on the coefficients α and β. α and β are lower in spring genotypes than winter genotypes, and they are nearly zero in absolutely spring genotypes not responding to vernalization, even having winter genotypes. In this study, the aforementioned equations were fitted to the experiment data for each genotype. Using cluster analysis through the Wards method, the obtained fitted α and β were then applied for grouping the studied wheat genotypes based on the intensity of the response to vernalization.

Data were processed with Excel, analyzed statistically with SAS, and cluster analysis with SPSS through the Wards method.

RESULTS AND DISCUSSION

The mean monthly temperature and mean monthly minimum temperature during the experiment at the experiment site was depicted in Fig. 1.



	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	Jun.
Aver.of month temp.	23	13.8	10.1	2.8	5.4	11.1	14.9	21.1	25.8
Aver.of min. temp.	12.7	4.4	1.0	-6.9	-2.9	2.0	7.6	13.3	17.6

Fig. 1. The mean monthly temperature and mean minimum temperature of air in South Khorasan Agricultural and Natural Resources Research Center, Birjand in 2013-2014.

Table 1: Variance analysis of traits in wheat genotypes under different vernalization treatments.

Source of variation	df	Mean of Square			
		Final leaf number	Days from transfer to flowering	Days from germination to flowering	Spike length
Vernalization (A)	4	32.86**	9940.99**	15389.97**	3.255**
Cultivars (B)	39	5.096**	276.01**	287.44**	4.011**
A*B	156	0.909**	375.9**	358.28**	2.184**
Error	200	0.367	3272	23322	82.956
C.V.		7.93	16.39	17.86	16.35

*, ** are significantly different at α = 0.05 and α = 0.01, respectively and ns is non-significant.

Table 2: Mean comparison of traits according to different vernalization treatments.

Vernalization time (Day)	Final leaf number	Days from transfer to flowering	Days from germination to flowering	Spike length
0	8.621 A	68.275 A	60.250 D	4.192 A
15	7.847 B	51.188 B	60.138 D	3.637 B
30	6.944 E	45.600 C	69.550 C	3.99 AB
45	7.267 D	43.463 C	81.263 B	3.98 AB
60	7.517 C	40.000 D	92.025 A	3.91 AB

Means in each row, followed by at least one letter in common are not significantly different at the 5% probability level using Duncan's Multiple Rang Test.

The minimum temperature in January was -6.9°C, which was 4.4°C during a thirty years period in the region. Analysis of variance showed the significant effect of vernalization duration and cultivars, as well as their interactions on all traits studied (Table 1). Comparison of the means showed that the transition period to the greenhouse until heading was reduced with increasing duration of vernalization. Vernalization duration had also a reverse effect on germination to flowering period (Table 2). The highest final leaf number was observed in the no- vernalization treatment. Spike length was also the longest in the no-vernalization treatments, and it had no significant difference among the other vernalization treatments (Table 2). Mean comparison of main stem final leaf number in these genotypes as affected by different vernalization treatments was bright in Table 3. The duration of vernalization fulfillment was about 30 days after planting at that year in the cultivars Gaspard, Karaj 3, Zare, Mihan, Dena, Soisons, Gaskogen, and Alvand. Also vernalization requirement of the cultivars Roshan, Bezostaya, Shahriar, and the lines C.86.5 was about 15 days after planting. The cultivar Azar 2 had the longest vernalization requirement of about 45 days after planting, (Fig. 2). Other cultivars had negligible vernalization requirements.

Fitting the equation of Wang *et al.* (1995) on the data of this study is shown in Table 4. Wang et al. suggested this equation to quantify the vernalization response of genotypes based on the effect of vernalization fulfillment on reducing the number of leaves. Some genotypes were true spring cultivars (such as Ghods, Parsi, Falat, Sepahan, Kavir, Maroon, WS-89-2, Darya, Ofogh, Nicknejad, Dez, Chamran, Atrak, Star, and Darab 2) which resulted in low coefficient of determination.

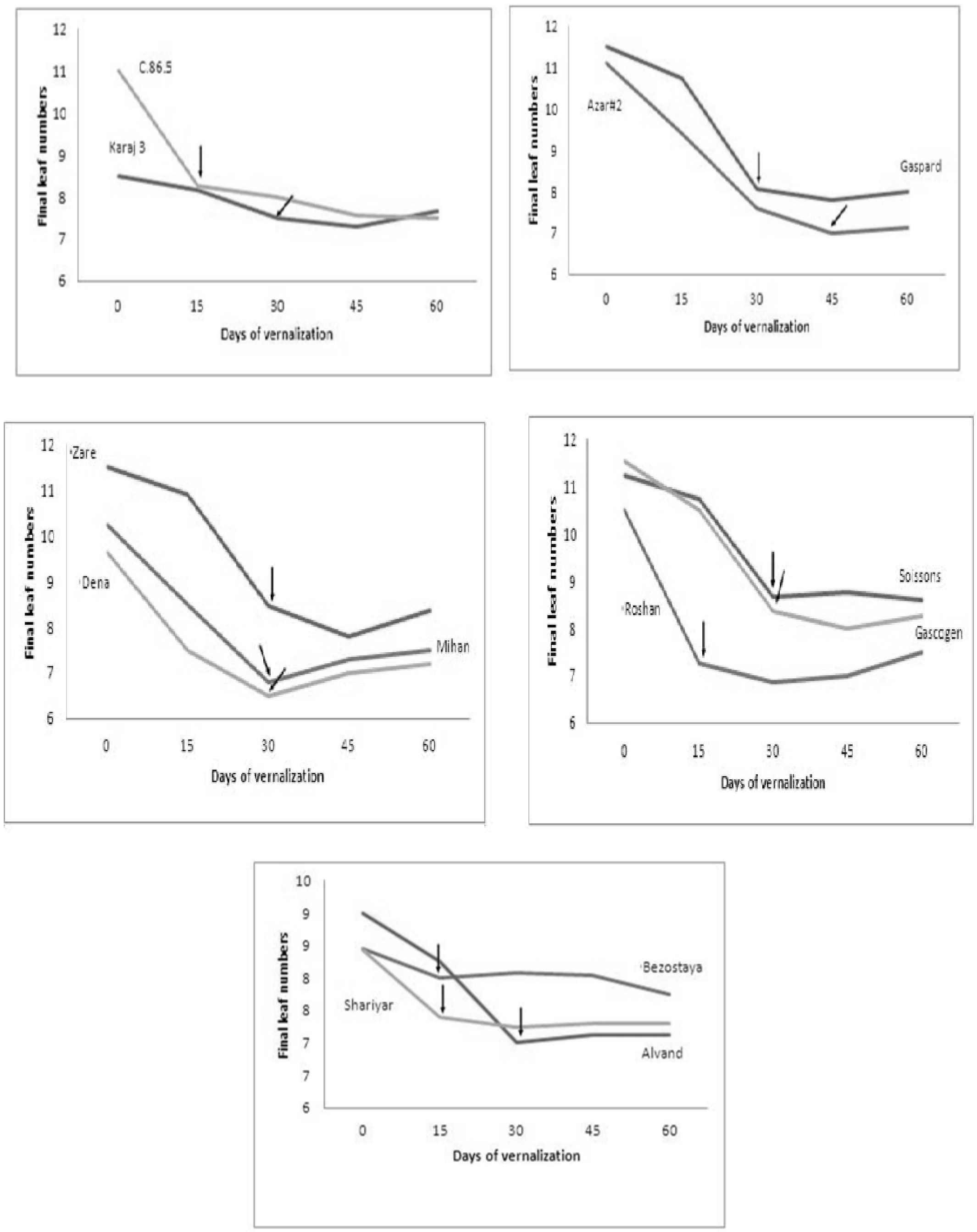


Fig. 2. Final leaf numbers of some of varieties that pass cooling required in field conditions and transfer to 16 hr. photoperiodic terms and 20± 2°C on 2013-2014 growing seasons. Arrows indicate the vernalization fulfillment point in each variety.

Table 3: Mean comparison of main stem final leaf number in bread wheat genotypes as affected by different vernalization treatments.

No.	Genotype	Vernalization treatments (day)				
		0	15	30	45	60
1	Bezostaya	8.450 LM	8.000 H	8.090 C	8.050 B	7.750 EF
2	Alvand	9.000 I	8.250 F	7.000 H	7.125 G	7.125 I
3	Ghods	7.150 T	7.650 J	6.750 J	7.250 F	7.550 HI
4	Shahriar	8.425 LM	7.400 L	7.250 G	7.500 D	7.300 K
5	Sepahan	7.375 R	7.125 NO	6.500 MN	7.000 H	7.000 M
6	Arta	8.750 J	7.125 NO	6.500 MN	7.300 F	6.500 O
7	Parsi	8.225 N	7.300 M	6.500 MN	8.000 B	7.750 EF
8	Falat	8.000 O	6.625 R	6.550 LM	7.000 H	7.800 E
9	Moghan 3	8.000 O	7.300 M	6.450 NO	7.000 H	7.150 I
10	Arg	7.375 R	7.000 P	6.800 IJ	7.000 H	7.000 M
11	Kavir	7.125 T	7.625 J	6.150 R	7.000 H	7.000 M
12	Maroon	6.600 V	7.250 M	6.500 MN	6.000 K	7.000 M
13	Roshan	10.500 E	7.250 M	6.875 I	7.000 H	7.500 IJ
14	Soissons	11.250 B	10.750 B	8.650 A	8.750 A	8.600 A
15	WS-82-9	6.950 U	8.000 H	6.250 Q	6.750 I	7.500 IJ
16	Gaskogen	11.550 A	10.500 C	8.375 B	8.000 B	8.250 C
17	Azar 2	11.100 C	9.400 D	7.600 E	7.000 H	7.125 I
18	Gaspard	11.500 A	10.750 B	8.050 CD	7.800 C	8.000 D
19	Darya	7.375 R	7.500 K	7.000 H	7.000 H	7.500 IJ
20	Zagros	8.625 K	7.150 N	6.625 KL	6.800 I	7.500 IJ
21	Tabasi	8.950 I	7.950 H	6.500 MN	7.000 H	6.750 N
22	Mahooti	9.250 H	6.600 R	5.750 T	6.500 J	7.000 M
23	Mihan	10.250 F	8.500 E	6.800 IJ	7.300 F	8.250 C
24	Zare	10.500 E	10.900 A	8.450 B	7.800 C	8.375 B
25	Dena	9.625 G	7.500 K	6.500 MN	7.000 H	7.625 GH
26	Sorkhtokhm	7.875 P	6.750 Q	5.900 S	6.750 I	6.750 N
27	Ofogh	8.750 J	7.250 M	6.250 Q	7.750 C	7.800 E
28	Nicknejad	7.375 R	7.250 M	6.400 O	7.000 H	7.650 G
29	Dez	8.375 M	7.500 K	7.000 H	7.000 H	8.250 C
30	Pishgam	10.250 F	8.500 E	7.000 H	7.000 H	8.250 C
31	Karaj 3	8.500 L	8.150 G	7.500 F	7.300 F	7.675 FG
32	Rasool	7.375 R	7.300 M	7.000 H	7.525 D	8.000 D
33	Chamran	8.250 N	7.050 OP	7.300 G	7.125 G	8.000 D
34	Pishtaz	8.500 L	7.500 K	6.800 IJ	7.125 G	6.750 N
35	Atrak	8.250 N	7.250 M	6.375 OP	7.000 H	7.600 GH
36	Darab 2	6.300 W	7.300 M	6.300 PQ	7.500 D	7.000 M
37	Star	7.625 Q	7.750 I	6.650 K	7.400 E	7.500 IJ
38	C.85.D.13	7.250 S	7.150 N	7.000 H	8.000 B	7.450 J
39	Morvarid	7.250 S	7.500 K	7.000 H	7.750 C	7.650 G
40	C.86.5	11.000 D	8.275 F	8.000 D	7.550 D	7.500 IJ

Means in each row, followed by at least one letter in common are not significantly different at the 5% probability using Duncan's Multiple Rang Test.

The results of grouping the studied cultivars and lines based on the coefficient α, indicating the potential of leaves at the beginning of insensitivity to vernalization are given in Fig. 3. Genotypes Soisons, Zare, C.86.5, Azar 2, Gaspard, and Gaskogen produced the highest number of leaves with more than 11 leaves in no vernalization requirement and were placed in a separate group.

Cultivars and lines Roshan, Pishgam, Mihan, Tabasi, Alvand, Dena, Bezostaya, and Karaj 3 were located in the group with intermediate production of leaves. In this regard, other cultivars with 6% similarity were placed in the lowest group (Fig. 4). According to this model, β indicates the degree of sensitivity to vernalization and equals to the exchange rate between vernalization days and the number of leaves.

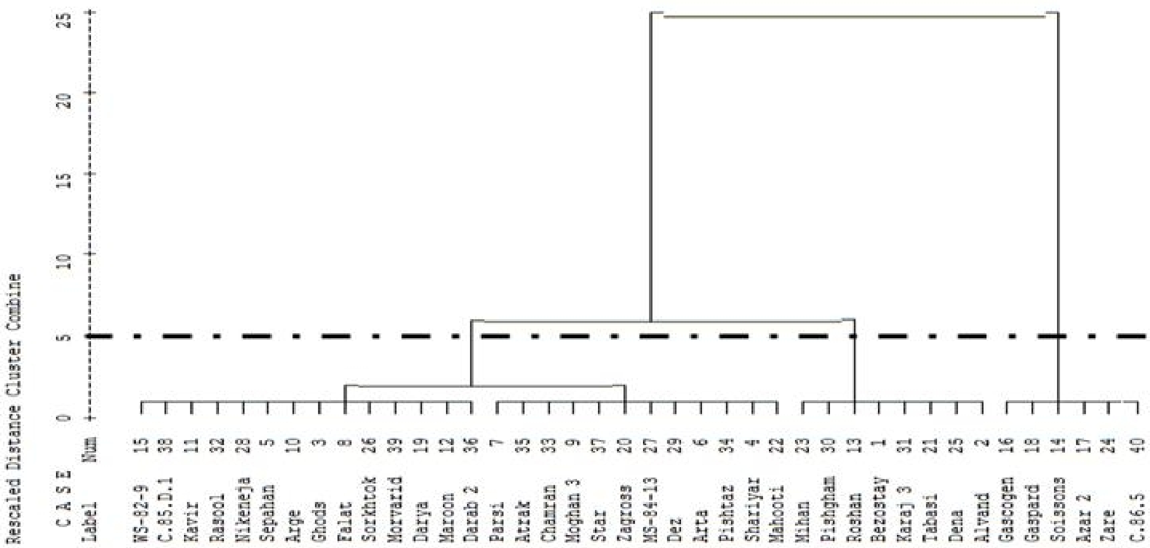


Fig. 3. The clustering of wheat genotypes based on α coefficient using Euclidean square distance and ward methods. Midline in this figure shows differences in the level of 6%.

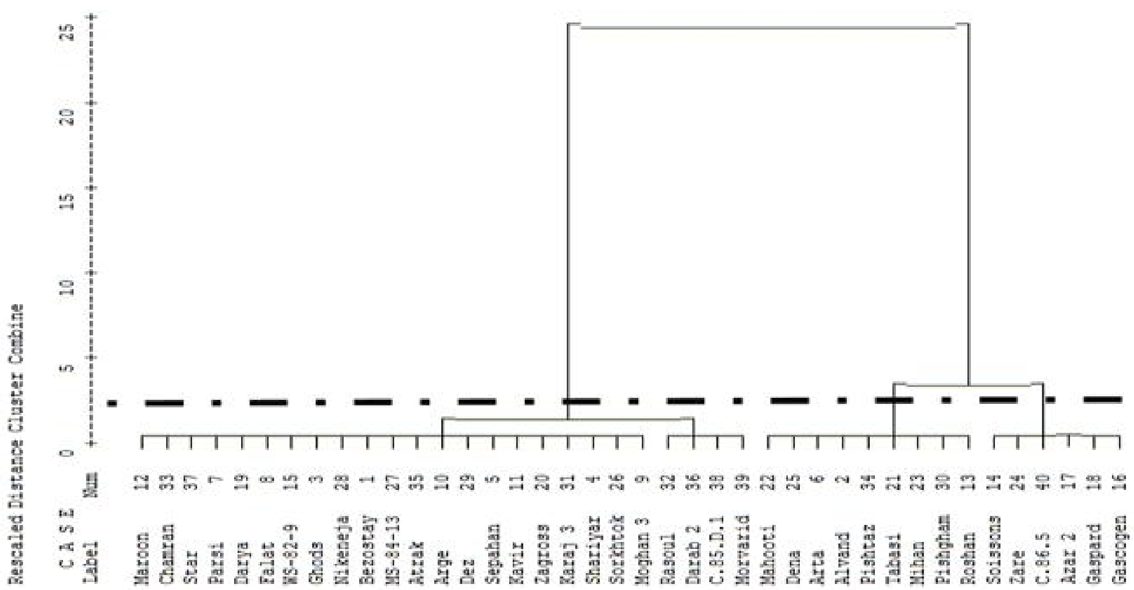


Fig. 4. The clustering of wheat genotypes based on β coefficient using Euclidean square distance and Ward methods. Midline in this figure shows differences in the level of 3%.

Table 4: Regression and determination coefficients for bread wheat genotypes fitting Wang *et al.*, (1995) model.

Cultivars	α	β	R^2
Bezostaya	8.338	0.009	0.922
Alvand	8.675	0.032	0.956
Ghods	7.190	-0.003	0.231
Shahriar	8.005	0.014	0.692
Sepahan	7.175	0.006	0.388
Arta	8.098	0.029	0.750
Parsi	7.605	0.002	0.203
Falat	7.200	0.000	0.200
Moghan 3	7.580	0.013	0.519
Arg	7.182	0.005	0.520
Kavir	7.155	0.006	0.268
Maroon	6.760	0.003	0.222
Roshan	9.075	0.042	0.626
Soissons	11.060	0.049	0.980
WS-82-9	7.120	0.001	0.201
Gaskogen	11.155	0.061	0.990
Azar 2	10.515	0.069	0.990
Gaspard	11.210	0.066	0.998
Darya	7.325	0.002	0.224
Zagros	7.860	0.017	0.469
Tabasi	8.500	0.036	0.899
Mahooti	7.940	0.031	0.501
Mihan	9.260	0.035	0.583
Zare	10.676	0.049	0.895
Dena	8.548	0.030	0.558
Sorkhtokhm	7.255	0.015	0.457
Ofogh	7.840	0.009	0.259
Nicknejad	7.075	-0.002	0.210
Dez	7.740	0.005	0.231
Pishgam	9.300	0.037	0.622
Karaj 3	8.325	0.017	0.848
Rasool	7.145	-0.010	0.604
Chamran	7.630	0.003	0.215
Pishtaz	8.110	0.026	0.930
Atrak	7.605	0.010	0.324
Darab 2	6.560	-0.011	0.405
Star	7.502	0.004	0.247
C.85.D.13	7.120	-0.008	0.459
Morvarid	7.220	-0.007	0.496
C.86.5	10.010	0.052	0.906

Similarly, grouping of the cultivars based on the coefficient α and the results of the genotypes grouping based on the coefficient β showed the highest sensitivity to vernalization in Soisons, Zare, C.86.5, Azar 2, Gaspard, and Gaskogen cultivars. Following one day reduction in vernalization fulfillment in these genotypes, 0.049-0.069 leaves decreased from the main stem daily. The genotypes Mahooti, Dena, Arta, Alvand, Pishtaz, Tabasi, Mihan, Pishgam, and Roshan had a moderate sensitivity to vernalization and other genotypes were placed in separate groups (Fig. 4). Under intermittent low temperature regimes, plants progress towards floral transition through a combination of the rates of progress achieved within and outside the vernalizing range of temperatures. This principle was able to explain to a reasonable extent the interchangeability of plant age and vernalization duration in reducing final leaf number that was observed by Wang *et al.* (1995).

With longer durations of low temperatures the vernalized condition became more and more insensitive to subsequent de-vernalizing temperatures until after 60 days at vernalization temperature was irreversible.

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