RESPONSE OF EUROPEAN BREAD WHEAT TO DIFFERENT VERNALIZATION TREATMENTS UNDER THE ENVIRONMENTAL CONDITIONS OF KURDISTAN-IRAQ

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Abstract. A factorial experiment was conducted in Kurdistan-Iraq to identify responses of European wheat varieties to vernalization treatments at 22ºC, 4ºC and -18ºC for two and four weeks in a Complete Randomized Block Design (CRBD) experiment with three replicates. Sabir-beg had the highest and most desired performance for most of the traits studied including grain yield. The highest spike and grain number were recorded for Hereford and Skalnige, respectively. Incubation temperatures had highly significant effects on most of the traits, including grain yield. Two weeks-vernalization at low temperature is satisfactory for vernalization of these wheat varieties. The interaction between varieties and incubation temperature produced varying effects. Grain number is mainly genotype-related. Sabir-beg×two weeks incubations had spent minimum days to flowering, followed by days to maturity. The varieties were persistent in their interaction with the incubation period. The interaction effect of 4ºC×four weeks incubation advanced flowering, extending flowering-maturation period to give a higher spike number. Harvest index, biomass, and grain yield were highly significant for both interactions of varieties×incubation temperature and varieties×incubation period. The three-factors interaction had a highly significant effect on most traits. The wheat varieties can be developed with more predictable responses to *in vitro* vernalization as an alternative to early sowing.
Keywords: *variety, in vitro, low temperature, VRN gene, grain yield*

Introduction

One of the critical stages in plant development is flowering, that affects crop production. The stage of crop vegetative/reproductive transition is a combination of plant responses to the environment including stresses (Kazan and Lyons, 2014). Optimal products can be obtained by the management of these stages. The time to flowering must coincide with favorable conditions to guarantee the species survival via producing viable seeds. Vernalization, a prolonged period of low temperature, is one of the environmental stimuli that is required to initiate flowering (Michaels, 2009; Dixon et al., 2019). Vernalization, as a plant cue, is required to transit the plant to reproductive stages, which means shifting from the vegetative growth to flowering and seed production (Trevaskis, 2010; Woods et al., 2019). This phenomenon is promoted by a chilling treatment (Chouard, 1960; Muterko and Salina, 2018). The effect of vernalization is to reduce the production period of leaf primordia (Griffiths et al., 1985), and consequently reduce the number of leaves and the tillers initiated on the main shoot (Gott et al., 1955; Levy and Peterson, 1972).

Winter cereals have a quantitative vernalization requirement for flowering (Woods et al., 2019). It has been identified that vernalized plants are more prone to flower earlier than non-vernalized (Robertson et al., 1996). Winter wheat genotypes grown under temperate regions can be vernalized at a seedling stage or even at full grain maturity, during the chilling period of winter (< 4°C) (Clay et al., 2012; Mureşan et al., 2019).

Bread wheat is one of the most cultivated grain crops occupying a wide range of lands worldwide, its winter varieties are planted in the autumn and they have adequate tolerance to survive freezing temperatures in winter. This adaptation occurs due to the reduction of moisture content and the accumulation of soluble carbohydrates in the crown area of the wheat plant, causing the reduction in growth rate at this stage, providing the plant resistance to frost temperature (Clay et al., 2012). Hence winter wheat varieties usually have higher yield potential than spring varieties planted later in the spring because of their longer growing period and vernalization explosion (Galiba et al., 2009; Li et al., 2013). Response to vernalization is one of the most important factors affecting environmental adaptation of wheat (Wang et al., 1995; Whittal et al., 2018). This action has been found to be under the effect of three genes of VRN1, VRN2, andVRN3, as a major gene for the vernalization in winter wheat. This was mapped using a population of recombinant inbred lines (RILs) generated from two winter wheat cultivars (Yan et al., 2006, 2015). After exposing the plants to non-freezing temperature for certain periods of time the cold-responsive genes are activated (Armonienė et al., 2013). Based on the exposing period of cold temperature to promote flowering on time, winter wheat has three categories; a weak winter type requires less than two weeks, a semi-winter type requires 2-4 weeks exposing of low temperature for flowering: while a strong type requires longer cold exposure (4 weeks and more) for timely flowering (Crofts, 1989). Flowering in wheat occurs after all main-stem leaves have appeared and are fully expanded. It is the consequence of the rate of leaf appearance which is controlled mostly by the temperature of the apical meristem and leaf expansion zones (McMaster and Wilhelm, 2003). *Triticum aestivum* L. (wheat, 2n = 6x = 42) is known to be cultivated across the most variable land area than any other grain crop (Rozbicki et al., 2019).

Vernalization requirement of winter wheat varies according to their genetic construction (Whittal et al., 2018). Varieties obtain sufficient vernalization when sown at an early time in autumn, while in case of late sowing with any reason, varieties will not start flowering on time (Mureşan et al., 2019), making the plant to have reduced flower or have late initiation, making the yield to be reduced quantitatively and qualitatively. The objective of this study was to identify the response of winter wheat varieties to different vernalization treatments in the control environment before planting, aiming at establishing the latest safe sowing date for winter wheat varieties, with a reasonable yield.

Materials and methods

A factorial experiment was conducted in the experimental field of the College of Agricultural Engineering Sciences, University of Sulaimani, Kurdistan of Iraq, located at Lat. 35° 34′ 307″; N, Long. 45° 21′ 992″; E, 765 m asl. CRBD experiment was conducted for five varieties, four European and one local variety (factor A), see *Table 1*.

Grains were treated with three different temperatures, namely at room temperature (22°C), at laboratory condition, 4°C (in a refrigerator) and at -18°C, in a freezer (factor B), for two different periods of two and four weeks (factor C). The incubation started on 12th November 2018 for four weeks incubation and on 27th November 2018 for two weeks incubation, to be directly sown on 12th December after termination of the designated period, see metrological data in *Table 2*. All treatment combination were sown in three replicates. Each replicate was applied on an area of 0.60 m^2, planting was performed on lines of two-meter length, distanced 30 cm between the lines, and 50 cm between replications.

Table 1. *Names and the sources of five wheat varieties used in the study*

No.	Name	Pedigree/origin	Source
1	Mariboss	Denmark	DCA - Danish Centre For Food And Agriculture, Aarhus University
2	Skalnige	Denmark	DCA - Danish Centre For Food And Agriculture, Aarhus University
3	Hereford	Denmark	DCA - Danish Centre For Food And Agriculture, Aarhus University
4	Ritraw	Denmark	DCA - Danish Centre For Food And Agriculture, Aarhus University
5	Sabir-beg	Local variety, Iraq	Bakrajo research Station, Sulaimani, Iraq

Table 2. *Meteorological data of Sulaimani- Iraq for the growing season 2018-2019*

Month	Temperature			Rainfall (mm)
	Min. Temp. (C°)	Max. Temp. (C°)	Average Temp. (C°)	
October 2018	9.6	32.0	19.2	48.2
November 2018	6.2	22.0	12.6	99.8
December 2018	-2.6	15.0	5.2	281.8
January 2019	0.8	13.8	5.4	210.6
February 2019	-2.0	17.4	6.8	108.2
March 2019	1.6	21.2	10.8	248.6
April 2019	2.4	26.0	13.2	190.0
May 2019	12.8	33.8	17.6	28.4
Total rainfall (mm)				1215.6

The land was tilled twice, smoothed, and plots were prepared before sowing. The varieties were sown on 12th December 2018 containing 30 treatment combinations in which they were homogenous experimental units. Grains size from all varieties was unified by sieving, to obtain persistent population density in the field. After incubation treatment for designated periods, the grains were planted within the lines for all replicates. Each replicate consisted of 3.5 g with uniform size.

The recommended doses of 80 kg N/ha and 80 kg P_2O_5/ha were applied for the experiment and all other agricultural practices were conducted as required. There was no chemical application (herbicide, herbicide, or pesticide) for the treatments, and the experiment was run under rainfed condition. Some physical and chemical properties of the experimental soil are given in *Table 3*.

Table 3. *Physical and chemical properties of the studied soil*

Soil properties	Soil texture (P.S.D)	Sand (g.kg^{-1})	Silt (g.kg^{-1})	Clay (g.kg^{-1})	E.C. (dS.m^{-1})	pH	O.M. (g.kg^{-1})	CaCO$_3$ (g.kg^{-1})	Total N (mg.kg^{-1})	K$^+$ (L^{-1})
Values	Clay	41.0	430.50	528.50	0.61	7.32	21.6	107.0	1.07	0.12

Note: E.C.: electronic conductivity; O.M.: organic matter; $CaCO_3$: Calcium Carbonate; K^+: potassium ion

The measurements of agro-morphological traits for growth characteristics, yield, and its components were recorded. Measured traits were:

- Plant Height: recorded at physiological maturity, height was recorded as the length in centimeters from the soil surface to the tip of spike excluding awns.
- Days to flowering: recorded as the number of days required from sowing till flower initiation.
- Days to maturity: recorded as the number of days required from sowing till physiological maturity.
- Spike number/m^2: calculated as a number of spikes per unit area (m^2).
- Grains/spike: counted as an average of the number of grains per spike for 5 selected spikes randomly per each plot.
- 1000- Kernel weight (g): one thousand grain weight (g) were taken at random from a bulk grain yield of each replicate.
- Biomass yield (ton/ha): recorded as the weight of all biological products of the plant above the soil surface per unit area.
- Harvest index (HI): Counted as a percentage of grain weight to biomass, according to the following equation (Shewry, 1998).

$$H.I. = \frac{\text{Grain yield}}{\text{biomass yield}} \qquad \text{(Eq.1)}$$

- Grain yield (ton/ha): calculated as a yield of grains of unit area, converted to ton per hectare.

Data were recorded for the agronomic traits, yield, and its components, and analyzed using XLSTAT 2016 software. The source of variance and comparison between the genotypes were determined. The comparisons of trait means were made using Duncan's multiple range test at 5% level of probability.

Results and Discussion

Studying phenological alteration such as vernalization is essential in winter wheat (*Triticum aestivum* L.), to cope with the adverse effects of global warming on crop production, especially under arid and semi-arid conditions (Shourbalal et al., 2019). It has been indicated that each degree increase in temperature results in the reduction of wheat production by 6% (Asseng et al., 2015). Advancing the process of flowering, through partial phenological alteration in winter wheat, is an important step to reduce the effect of drought (Searle and Coupland, 2004). To induce yield production of winter wheat in mild and dried condition, a period of vernalization is required to initiate reproductive stage following vegetative growth (Heide and Sønsteby, 2015).

The analyzed results indicated a significant variance of the genotypes and their interaction treatments with variable incubation temperature and the exposure period to the incubated temperature (*Table 4*). Vernalization determines flowering and heading times, giving a significant influence on the adaptability of wheat plants to different environmental conditions, that mainly contribute to establishing yield and yield components (Slafer, 2003; Chen et al., 2013). Varieties had a highly significant variance for all the studied traits. The levels of incubation temperature had highly significant effects on days to maturity, spike number/m^2, grain number/spike, 1000 kernel weight, harvest index, and grain yield, while they had a significant effect on days to flowering.

Table 4. *Analysis of variance for agronomic traits, yield and its components of wheat varieties, incubation temperature and the period of incubation with their interaction effects*

Source	df	Mean square								
		Plant height (cm)	Days to flowering	Days to maturity	Spike number/m^2	Grain number/spike	1000 kernel weight (g)	Harvest index	Biomass yield (ton/ha)	Grain yield (ton/ha)
Varieties (F1)	4	5293.328**	3195.711**	3678.933**	31225.483**	947.763**	441.031**	0.006**	77.354**	6.612**
Incubation temperature (F2)	2	5.204	7.878*	24.1**	4107.011**	128.795**	19.313**	0.018**	0.644	3.133**
Period of Incubation (F3)	1	8.465*	0.178	33.611*	6.4	2.347	15.528	0.00037	1.686	0.015
Replicates	2	15.481**	1.078	1.9	49.244	4.065	1.801	0.001	1.126	0.037
Factor1*Factor2	8	13.498**	7.919**	14.517**	10485.858**	107.45**	16.118**	0.002**	0.965	0.707**
Factor1*Factor3	4	6.787**	2.567	3.556*	1768.761*	20.051*	24.649**	0.011**	3.193**	1.045**
Factor2*Factor3	2	4.238	12.811**	14.144**	291.433	2.028	49.784**	0.016**	4.313**	3.386**
Factor1*Factor2*Factor3	8	6.103**	20.992**	10.172**	3547.253**	27.449**	22.818**	0.005**	4.638**	0.732**
Error	58	105.445	1.561	0.992	570.474	9.122	0.924	0.0003	0.614	0.015
Minimum		70.5	101	145	232	17.6	15.352	0.14	7.066	1.589
Maximum		117.5	143	182	484	55.75	33.992	0.341	16.813	4.968
Mean		84.378	125.644	170.367	350.822	32.989	23.568	0.248	12.52	3.18
Std. deviation		15.55	12.159	13.025	56.71	8.039	5.177	0.048	2.163	0.794

Period of incubation for different temperatures had no significant effect on the studied traits, except plant height and days to maturity. The interaction of varieties×incubation temperature had a highly significant effect on all traits except for biomass yield. Distribution of the bio-product between sink and source for the wheat varieties was not influenced by the period of incubation. Both interactions of varieties×incubation temperature and varieties× incubation period had similar effects on the studied traits, as harvest index, biomass yield, plant height, and grain yield, and 1000 kernel weight were highly significant for both interactions. A very close pattern was also stated for the interaction between the incubation temperature and the incubation period. The interaction of all three factors showed a highly significant effect on all traits except grain number/spike.

Wheat varieties had a considerable variable effect on the studied characteristics (*Table 5*). Plant height tends to be the highest in Sabir-beg variety (114.861 cm), while other varieties have a close range of height with each other (only 4 cm differences), their values are ranging from 73.8-78,833cm for Maribos and Ritraw, respectively. The local variety (Sabir-beg) spent shorter period to start flowering (101.94 days from sowing), it was earlier in flowering as well than Hereford with a total of 28 days. The same trend was obtained for days to maturity for Sabir-beg and Hereford, respectively. Maximum days required to maturity was referred to Mariboss, spending 179.7 days after sowing. Hereford had the highest number of spike per unit area (413.667 spike/m^2), while the least number of spikes was referred to Mariboss.

Table 5. *Effect of varieties on agronomic traits, yield and its components of wheat varieties*

Varieties	Plant height (cm)	Days to flowering	Days to maturity	Spike number/m²	Grain number/ spike	1000 kernel weight (g)	Harvest index	Biomass yield (ton/ha)	Grain yield (ton/ha)
Mariboss	73.807 d	133.833 a	179.722 a	311.889 d	28.983 d	22.822 c	0.250 bc	11.701 c	2.905 c
Skalnige	76.370 c	130.778 bc	177.167 b	339.833 c	40.556 a	19.370 d	0.262 ab	10.069 d	2.738 d
Hereford	78.017 b	130.056 c	174.167 d	413.667 a	34.519 c	19.330 d	0.248 c	12.678 b	3.363 b
Ritraw	78.833 b	131.611 b	175.722 c	369.556 b	38.256 b	25.019 b	0.219 d	12.395 b	2.733 d
Sabirbeg	114.861 a	101.944 d	145.056 e	319.167 d	22.633 e	31.298 a	0.264 a	15.756 a	4.162 a

In terms of grain number/spike the highest number of 40.566 grain was recorded for Skalnige variety, followed by Ritraw, however the least number of grain (22.633) was recorded for Sabir-beg variety. A wide range of 1000 kernel weight was also observed for the varieties under study. The maximum of 31.298 g was recorded for Sabir-beg, while the lowest value of 1000 kernel weight was recorded for Hereford and Skalnige, respectively, with nearly 11 g less weight than Sabir-beg. Harvest Index was also significant for the varieties studied. Sabir-beg had the highest harvest index of 0.264, followed by Skalnige, while the lowest harvest index referred to Ritraw. Biomass was also indicated the variation between the varieties under study. Sabir-beg had indicated the highest biomass yield of 15.756 ton/ha, while the minimum of 10.069 ton/ha was recorded for Skalnige. The same pattern of superiority in the grain yield was recorded for Sabir-beg giving a total of 4.162 ton/ha grain yield. The superiority of local wheat varieties to most of the newly introduced genotypes in another study by Ali et al. (2018) compared to some European varieties for the yield and its component highly supports the current result. The genetic variations that drive a wide response of the genotype understudy could serve the development of a new cultivar with high resilience to future climate changes through the introgression of important traits.

The levels of temperature treatment had variable effects on the studied traits (*Table 6*). Plant height had no significant difference for different temperature levels. Days to flowering was affected with -18°C incubation, however the incubation at 4°C had no significant effect on flowering day. Vernalization sensitivity of some of the wheat varieties could be the reason for delaying flowering in certain environmental conditions (Ortiz-Ferrara et al., 1995), making the temperature level of 4°C to be with less effect to flowering compared to the normal ambient temperature of 22°C. The same pattern was also observed for days to maturity, spending less days to maturity (166.333 days) for -18°C incubation. A positive effect of vernalization on days to flowering has been confirmed by other researchers (Sharma et al., 2012). This trend indicates the effect of low-temperature incubation on the vernalization of the varieties under study, giving the desired signal to vegetation period for some days, allowing plants to shift to flowering and maturity period earlier. Hence, in case of impossibility of sowing in the normal sowing times in autumn or at the beginning of winter, the requirement of vernalization could be afforded *in vitro* as a substitute to early sowing. In terms of the spike number per unit area, the low temperature of 4°C had a significant effect on the spike number (364.233 spike/m^2).

Table 6. *Effect of incubation temperature on agronomic traits, yield and its components of wheat varieties*

Temperature	Plant height	Days to flowering	Days to maturity	Spike number/m^2	Grain number/ spike	1000 kernel weight (g)	Harvest index	Biomass yield (ton/ha)	Grain yield (ton/ha)
22 °C	84.266 a	125.300 a	170.833 a	345.533 b	35.297 a	23.701b	0.268 a	12.669 a	3.475 a
4 °C	84.839 a	125.400 a	170.933 a	364.233 a	32.383 b	22.707 c	0.256 b	12.376 a	3.232 b
-18 °C	84.029 a	122.233 b	166.333 b	342.700 b	31.288 b	24.295 a	0.221 c	12.514 a	2.835 c

A reverse effect was recorded for the vernalization temperatures on the number of grains per spike, as the increased chilling temperature has negatively reduced the grain number/spike. There were also significant differences in the levels of incubation temperature for 1000 kernel weight. Decreasing the temperature to 4°C had a negative effect on the weight of kernels, while a further reduction in the incubation temperature to -18°C had increased the weight of 1000 kernels (24.295 g). Harvest index was negatively affected due to the reduced temperature. Total biomass was not affected by different incubation levels. The accumulation of bio-product is more genetically controlled (Kumar et al., 2016; Brinton et al., 2017), while tracking the product to be accumulated to different parts of the plant is a matter of environmental effect in addition to genetic structure. Grain yield was identified to be reduced by decreasing the incubation temperature.

The incubation period for the wheat varieties seems to have a high effect on plant height and days to maturity (*Table 7*). Plant height had a few increases with the extended incubation period, while days to maturity has decreased with the same trends of incubation extension. No significant increase in grain weight was realized by increasing the period of incubation from two to four weeks duration. This result indicates the satisfaction period of two weeks incubation at low temperature for the wheat varieties under study. The varieties under study might be weak winter genotypes and the 2 weeks duration could be satisfactory for vernalization as extending to four weeks duration of a cold explosion being non-necessary for the current genotypes.

Table 7. *Effect of incubation period on agronomic traits, yield and its components of wheat varieties*

Period of incubation	Plant height (cm)	Days to flowering	Days to maturity	Spike number/m2	Grain number/ spike	1000 kernel weight (g)	Harvest index	Biomass yield (ton/ha)	Grain yield (ton/ha)
Two weeks	84.071 b	169.756 a	13.383 a	351.089 a	33.151 a	23.153 a	0.250 a	12.383 a	3.193 a
Four weeks	84.984 a	170.978 a	11.657 b	350.556 a	32.828 a	23.983 a	0.247 a	12.657 a	3.167 a

The interaction of varieties×incubation temperature for the grain of wheat varieties had different effects on the studied characteristics (*Table 8*). The interaction between Sabir-beg and 4ºC gave the maximum plant height of 115.583 cm, while the minimum plant height of 72.078 cm was referred to as the interaction between Mariboss and 22ºC incubation. Same interaction effect of Sabir-beg×4ºC was recorded for minimum days required to flowering (101.667 days). The interaction of Sabir-beg variety to other incubation temperatures had the same effect on the days to flowering. The maximum number of days required to start flowering (131.5 days) was recorded for the interaction between Mariboss and 22ºC incubation. Like days to flowering the minimum days to maturity was referred to Sabir-beg interaction with different incubation temperatures, requiring 145 days to maturity, however, the maximum days spent to maturity was recorded for Mariboss with the incubation of 22ºC×4ºC. The maximum spike number of 442 spike/m^2 was identified for the interaction of Hereford×22ºC incubation, followed by the interaction of the same variety with -18ºC. The minimum number of spikes/m^2 was recorded for Mariboss interacted with 22ºC incubation. Grain number is one of the yield component traits that was recorded as a maximum of 49.667 grains/spike for the interaction of Skalnige variety with 22ºC incubation.

Table 8. *Interaction effect of varieties× incubation temperature on agronomic traits, yield and its components of wheat varieties*

Varieties× incubation temperature	Plant Height (cm)	Days to flowering	Days to maturity	Spike number/m^2	Grain number/ spike	1000 kernel weight (g)	Harvest index	Biomass yield (ton/ha)	Grain yield (ton/ha)
Mariboss×22ºC	72.078 h	131.500cde	180.833 a	257.000 k	26.400 f	22.235 fg	0.240 efg	11.886 a	2.838 fg
Mariboss×4ºC	73.155 gh	133.833 b	180.667 a	347.667 efg	30.217 e	22.484 f	0.280 abc	11.393 a	3.140 e
Mariboss× -18ºC	76.189 f	136.167 a	177.667 b	331.000 fgh	30.333 e	23.747 e	0.229 g	11.824 a	2.737 g
Skalnige×22ºC	76.833 ef	130.333cef	179.667 a	359.000 def	49.667 a	19.869 h	0.272 bcd	10.133 a	2.758 fg
Skalnige×4ºC	77.989cde	130.167 ef	177.667 b	316.833 hij	37.833 c	17.133 j	0.257 def	10.067 a	2.558 h
Skalnige× -18ºC	74.289 g	131.833 cd	174.167cd	343.667efgh	34.167 cd	21.108 g	0.256 def	10.006 a	2.900 f
Hereford×22ºC	78.083cde	130.333def	174.000cd	442.000 a	33.233 de	18.696 i	0.284 ab	12.300 a	3.818 c
Hereford×4ºC	78.883 c	130.000 ef	173.667 d	380.333 cd	37.033 cd	19.919 h	0.252 defg	12.427 a	3.505 d
Hereford× -18ºC	77.083 def	129.833 f	174.833cd	418.667 ab	33.289 de	19.374 hi	0.207 h	13.307 a	2.767 fg
Ritraw×22ºC	79.167 c	132.000 c	174.667cd	381.667 cd	42.633 b	27.227 c	0.244 efg	12.958 a	3.169 e
Ritraw×4ºC	78.583 cd	131.333cdef	177.500 b	406.667 bc	35.917 cd	21.816 f	0.234 fg	12.362 a	2.895 fg
Ritraw×-18ºC	78.750 cd	131.500cdef	175.000 c	320.333 ghi	36.217 cd	26.015 g	0.180 i	11.866 a	2.134 i
Sabir-beg×22ºC	115.167ab	102.333 g	145.000 e	288.000 j	24.550 fg	30.477 b	0.298 a	16.069 a	4.790 a
Sabir-beg×4ºC	115.583 a	101.667 g	145.167 e	369.667 de	20.917 g	32.185 a	0.260 cde	15.633 a	4.062 b
Sabir-beg× -18ºC	113.833 b	101.833 g	145.000 e	299.833 ij	22.433 g	31.233 ab	0.233 fg	15.565 a	3.634 d

The minimum grain number/spike referred to the interaction of Sabir-beg with all incubation treatments. From this result, it seems that the grain number trait is mainly genotype-related (Guo et al., 2015), as the incubation treatment has no significant effect on the improvement of this trait. The interaction of Sabir-beg with the 4°C and -18°C incubations showed the maximum weight of 1000 kernel (32.185 g and 31.233 g, respectively). Skalnige interacting with 4°C incubation had the minimum of 1000 kernel weight (17.1333 g). The interaction of Sabir-beg with the 4°C had the maximum record for harvest index, biomass yield and grain yield, while the minimum record refer to Ritraw interacting with -18°C incubation for both traits of harvest index and grain yield. It is clear that Sabir-beg is the most adapted variety to the local environment, requiring less vernalization, which makes the interaction of this variety with low incubation temperatures to give less effect. In terms of biomass yield the minim value (however not significant) referred to the interaction of Skalnige with all incubation temperatures. Less vernalization effect on the European varieties characteristics in this study could refer to the presence of allelic variation (VRN) at vernalization (Whittal et al., 2018). Presence of another allele (VRN4) or recessive VRN allele to VRNI gene could drive the transcription of this gene without severe needs for vernalization period (Goncharov, 2004; Kippes et al., 2015), or they might be semi-winter cultivars that require less period of low incubation temperature for full vernalization, and capable of switching to generative development under local environmental conditions (Goncharov, 2004; Li et al., 2013). Another possibility is that, planting the current varieties by mid of December did not allow the germinated plant to catch enough short day exposure, as a short day phototropism combined with low temperatures is required to permit VRN1 expression for floral transition of the meristem at an earlier period (Hemming et al., 2008; Li and Dubcovsky, 2008; Whittal et al., 2018), as a result no realized differences between the treated vernalization and controlled wheat grains were recorded.

The interaction between varieties and incubation periods had different effects on the studied characteristics (*Table 9*). Plant height had the maximum value of 115.722 cm for the interaction between Sabir-beg and four weeks incubation, followed by the interaction Sabir-beg×two weeks incubations. A minimum plant height was referred to as Mariboss interacted with four weeks incubations. The same interaction of Sabir-beg×2 weeks incubation had spent the minimum days to start flowering (101.778). Regarding plant height, the maximum days required to start flowering was recorded for Mariboss interacting with two and four weeks incubations. The same pattern was also realized for days to maturity. Spike number was identified to be at the maximum rate (423.00 spike/m^2) for Hereford interacting with two weeks incubation, while the least value was recorded for the interaction of Mariboss×2 weeks incubation, giving 296.444 spike/m^2.

A maximum number of 40.978 and 40.133 grain/spike was identified for Skalnige interacting with two and four weeks incubations, while the least number of 22.39 grains/spike referred to Sabir-beg interacting with two weeks interaction. The highest 1000 kernel weight was 31.488 g and 31.109 g for Sabir-beg interacting with two and four weeks incubations, respectively. While the interaction of Hereford with four weeks incubation had the lowest weight of 18.506 g for 1000 grain of wheat. Harvest index was identified to be at a maximum rate of 0.289 for Mariboss interacting with four weeks of incubation. Biomass yield had the maximum value of 15.773 and 15.739 ton/ha for Sabir-beg variety interacting with two and four weeks of incubation, respectively. A minimum value of 9.231 ton/ha was recorded for Skalnige interacting with two weeks incubation. Grain yield also had the same pattern as biomass yield, giving the maximum

yield of 4.197 and 4.127 ton/ha for the interaction of Sabir-beg with two and four weeks of incubation, respectively. The minimum grain yield was recorded for Mariboss interacting with 2 weeks incubation. The results here indicated the superiority of Sabir-beg interacting with both incubation periods, which surpassed other varieties in the majority of traits. The persistence of the genotypes is a major insight for this experiment, as the incubation of four weeks period had no significant change compared to the two weeks incubation.

Table 9. Effect of varieties and period of storage interaction on agronomic traits, yield and its components of wheat varieties

Varieties × Incubation period	Plant height (cm)	Days to flowering	Days to maturity	Spike number/m²	Grain number/ spike	1000 kernel weight (g)	Harvest index	Biomass yield (ton/ha)	Grain yield (ton/ha)
Mariboss×2 weeks incubation	74.352 f	133.333 a	178.889 b	296.444 f	30.867e	20.521d	0.210 e	11.954cd	2.522 f
Mariboss×4 weeks incubation	73.263 f	134.333 a	180.556 a	327.333 de	27.100 f	25.123bc	0.289 a	11.449de	3.289 c
Skalnige×2 weeks incubation	75.859 e	131.222 a	176.111 c	347.000 cd	40.978 a	19.076 ef	0.281ab	9.231 f	2.838 e
Skalnige×4 weeks incubation	76.881de	130.333 a	178.222 b	332.667 de	40.133ab	19.664 de	0.242 d	10.906 e	2.638 f
Hereford×2 weeks incubation	77.200 d	130.333 a	173.778 e	423.000 a	33.815 d	20.154 d	0.264bc	12.496bc	3.578 b
Hereford×4 weeks incubation	78.833 c	129.778 a	174.556de	404.333 a	35.222cd	18.506 f	0.232 d	12.859 b	3.149 d
Ritraw×2 weeks incubation	78.944 c	131.778 a	174.889 d	372.556 b	37.700bc	24.524 c	0.232 d	12.460bc	2.902 e
Ritraw×4 weeks incubation	78.722 c	131.444 a	176.556 c	366.556 bc	38.811ab	25.514 b	0.207 e	12.330bc	2.563 f
Sabir-beg×2 weeks incubation	114.000b	101.778 a	145.111 f	316.444 ef	22.394 g	31.488 a	0.261c	15.773 a	4.127 a
Sabir-beg×4 weeks incubation	115.722a	102.111a	145.000 f	321.889 e	22.872 g	31.109 a	0.266bc	15.739 a	4.198 a

The effect of different vernalization temperatures and the period of incubation is clarified in *Table 10*. The interaction between incubation temperature and the period of incubation was not significant however the highest plant height of 85.44 cm was recorded for 4°C×4 weeks incubation. The same interaction was significant for days to flowering, recording the least days spent to flowering, while it had the highest and most significant number of days to maturity. It seems from this result that the interaction effect of 4°C incubation with four weeks period had allowed the flowering to be advanced, allowing the plant to stay longer in the flowering stage giving a higher number of spike per unit area with reasonable biomass. Proper management of the flowering period results in higher productivity, however, the highest yield of 3.584 has resulted from the interaction of 4°C×2 weeks incubation. Significant effect of vernalization in wheat has been investigated previously at the temperature below 10°C (Robertson et al., 1996).

Same interaction of 4°C×4 weeks incubation showed the highest potential of 365.333 spikes per square meter, however it is not statistically significant. Grain number/spike was also not significant for the interaction between the incubation temperature and the period of incubation, while the maximum value of 35.43 grain/spike was recorded for the interaction between 22°C×2 weeks incubation. The interaction

of -18°C×4 weeks incubation showed the highest value of 26.182 g for 1000 kernel weight. The interaction of 4°C×2 weeks incubation gave the highest harvest index of 0.281, while the lowest value referred to the interaction of -18°C×2 weeks incubation. Biomass yield recorded the maximum value of 13.007 followed by 12.931 ton/ha for the interaction of -18°C×4 weeks incubation and 22°C×2 weeks incubation, respectively. Grain yield was identified to reach a maximum of 3.584 ton/ha for the interaction of 4°C×2 weeks incubation, the lowest grain yield of 2.515 ton/ha has been identified for the interaction between -18°C and 2 weeks incubation. This combination treatment between different incubation temperatures and their periods could explore the efficiency of 4°C with two weeks incubation could be satisfactory to induce the vernalization in the wheat varieties under study, as any further extension in the duration of cold treatment would not affect a reduction in the flowering period (Kim et al., 2009).

Table 10. *Effect of temperature and period of storage interaction on vegetative growth, yield and its components of wheat varieties*

Temperature × incubation period	Plant Height (cm)	Days to Flowering	Days to maturity	Spike number/m²	Grain number/ Spike	1000 kernel weight	Harvest index	Biomass yield	Grain yield
22°C×2 weeks incubation	84.382 a	125.267bc	170.867 b	343.600 a	35.430 a	23.834b	0.267 a	12.931 a	3.481b
22°C×4 weeks incubation	84.149 a	125.333bc	170.800 b	347.467 a	35.163 a	23.568b	0.268 a	12.408ab	3.468b
4°C×2 weeks incubation	84.238 a	126.133ab	169.600 c	363.133 a	32.300 a	23.215b	0.281 a	12.197 b	3.584 a
4°C×4 weeks incubation	85.440 a	124.667 c	172.267 a	365.333 a	32.467 a	22.200c	0.232 b	12.556ab	2.880d
-18°C×2 weeks incubation	83.593 a	125.667 b	168.800 d	346.533 a	31.722 a	22.408c	0.201 c	12.021 b	2.515 e
-18°C×4 weeks incubation	84.464 a	126.800 a	169.867 c	338.867 a	30.853 a	26.182a	0.242 b	13.007 a	3.154 c

There are variable effects on all the traits with the triple interaction effect of varieties×temperature×incubation period (*Table 11*). Sabir-beg interacting with the other two factors showed the best records for the traits plant height, days to flowering, days to maturity, 1000 kernel weight, biomass and grain yield. The reason for surpassing Sabir-beg by the other genotypes either individually or interacting with other factors could refer to the genetic architecture of these genotypes to validate VRN1 alleles to reduce or eliminating the vernalization requirement and able to manage the vernalization stage in a few days at a suitable temperature ranged from 5-20°C (Mureşan et al., 2019). Hereford×4°C×two weeks incubation had a reasonable grain yield after Sabir-beg, recoding 4.546 ton/ha, also the maximum harvest index of 0.327 was recorded for it.

Despite the above results for the interaction of genotypes, incubation temperature, and its period, many other genotypic and environmental factors could be involved in this highly complex biological process. It is identified that mechanisms of vernalization are not just genetically controlled, they are also categorized based on their interactions with the environment (Griffiths et al., 2009). Despite the complexity of genetic and environmental interaction in vernalization operation, other genes are also involved in relation to vernalization, such as VER2 (Yong et al., 2003), which encourage de-vernalization in wheat by silencing the activity of VRN1 gene.

Table 11. *Effect of the interaction between varieties, temperature and period of storage on vegetative growth, yield and its components of wheat varieties*

Varieties × Temperature × Period of incubation	Plant height	Days to flowering	Days to maturity	Spike number/unit area	Grain number/ Spike	1000 kernel weight	Harvest index	Biomass yield	Grain yield
Mariboss× 22 ˚C × Two weeks	72.578 jk	131.333 cde	181.000 a	252.000 k	26.400 ijk	22.402 ef	0.237 ijkl	12.053 efgh	2.855 jkl
Mariboss × 22 ˚C × Four weeks	71.578 k	131.667 cd	180.667 a	262.000 k	26.400 ijk	22.069 efg	0.243 hijkl	11.720 efghi	2.821 jkl
Mariboss × 4 ˚C × Two weeks	72.589 jk	137.000 b	180.333 a	341.333 fghi	30.533 hi	21.593 fgh	0.243 hijkl	12.369 cdefg	2.999 ijk
Mariboss × 4 ˚C × Four weeks	73.722 ijk	130.667 de	181.000 a	354.000 defgh	29.900 hij	23.374 de	0.317 ab	10.417 ijk	3.282 gh
Mariboss × -18 ˚C × Two weeks	77.888 cde	131.667 cd	175.333 b	296.000 Jk	35.667 efgh	17.568 lm	0.151 o	11.440 fghij	1.712 o
Mariboss × -18˚C× Four weeks	74.489 hij	140.667 a	180.000 a	366.000 defgh	25.000 jkl	29.925 b	0.308 abc	12.209 efgh	3.763 e
Skalnige × 22˚C × Two weeks	77.000 defg	130.333 de	180.000 a	374.000 defg	52.000 a	19.869 hijk	0.272 defghi	10.133 jk	2.758 l
Skalnige × 22˚C × Four weeks	76.667 efgh	130.333 de	179.333 a	344.000 efghi	47.333 ab	19.869 hijk	0.272 defghi	10.133 jk	2.758 l
Skalnige × 4˚C × Two weeks	77.333 cdefg	130.000 de	174.333 bc	340.333 ghi	37.133 defg	16.405 m	0.293 abcdef	9.373 kl	2.739 l
Skalnige × 4˚C × Four weeks	78.644 cde	130.333 de	181.000 a	293.333 jk	38.533 cde	17.860 lm	0.220 lm	10.760 hijk	2.376 m
Skalnige × -18˚C × Two weeks	73.244 ijk	133.333 c	174.000 bc	326.667 hij	33.800 efgh	20.953 fghi	0.278 cdefgh	8.186 l efghi	3.018 ij
Skalnige × -18˚C × Four weeks	75.333 fghi	130.333 de	174.333 bc	360.667 defgh	34.533 efgh	21.263 fgh	0.235 jkl	11.826 bcd	2.781 kl
Hereford × 22˚C × Two weeks	77.833 cdef	130.333 de	174.000 bc	422.000 abc	31.733 ghi	19.196 ijkl	0.279 cdefg	13.800 hijk	3.835 de
Hereford × 22˚C × Four weeks	78.333 cde	130.333 de	174.000 bc	462.000 a	34.733 efgh	18.196 kl	0.289 bcdef	10.800 ghij	3.801 de
Hereford × 4˚C × Two weeks	78.600 cde	130.000 de	173.333 c	398.667 bcd	37.533 cdefg	20.897 fghi	0.327 a	10.911 b	4.546 b
Hereford × 4˚C × Four weeks	79.167 cde	130.000 de	174.000 bc	362.000 defgh	36.533 efg	18.941jkl	0.177 no	13.943 bcdef	2.464 m
Hereford × -18˚C × Two weeks	75.167 ghi	130.667 de	174.000 bc	448.333 a	32.178 fgh	20.368 ghij	0.185 n	12.778 bc	2.353 m
Hereford × -18˚C × Four weeks	79.000 cde	129.000 e	175.667 b	389.000 bcde	34.400 efgh	18.380 kl	0.230 jkl	13.835 bcdef	3.182 hi
Ritraw × 22˚C × Two weeks	79.500 cd	132.000 cd	174.333 bc	380.000 cdefg	42.467 bcd	27.227 c	0.247 ghijkl	12.791 bcde	3.169 hi
Ritraw × 22˚C × Four weeks	78.833 cde	132.000 cd	175.000 bc	383.333 cdefg	42.800 bc	27.227 c	0.241 ijkl	13.124 cdefg	3.169hi
Ritraw × 4˚C × Two weeks	77.500 cdefg	132.000 cd	174.667 bc	384.000 cdefg	33.800 efgh	24.457 d	0.284 bcdef	12.386 defg	3.515 f
Ritraw × 4˚C × Four weeks	79.667 c	130.667 de	180.333 a	429.333 ab	38.033 cdef	19.175 ijkl	0.183 n	12.337 efgh	2.275 m
Ritraw × -18˚C × Two weeks	79.833 c	131.333 cde	175.667 b	353.667 defgh	36.833 efg	21.888 efg	0.166 no	12.204 fghij	2.023 n
Ritraw × -18˚C × Four weeks	77.667cdefg	131.667 cd	174.333 bc	287.000 jk	35.600 efgh	30.141 b	0.195 mn	11.528 a	2.245 m
Sabir-beg × 22˚C × Two weeks	115.000 a	102.333 f	145.000 d	290.000 jk	24.550 jklm	30.477 b	0.301 abcd	15.878 a	4.790 a
Sabir-beg × 22˚C ×Factor-3-C2	115.333 a	102.333 f	145.000 d	286.000jk	24.550 jklm	30.477 b	0.294 abcde	16.261 a	4.790 a
Sabir-beg × 4˚C × Two weeks	115.167 a	101.667 f	145.333 d	351.333 efghi	22.500 klm	32.721 a	0.258 fghijk	15.946 a	4.121 c
Sabir-beg × 4˚C × Four weeks	116.000 a	101.667 f	145.000 d	388.000 bcdef	19.333 m	31.648 ab	0.261efghij	15.320 a	4.004 cd
Sabir-beg × -18˚C × Two weeks	111.833 b	101.333 f	145.000 d	308.000 ij	20.133 lm	31.264 ab	0.224 klm	15.495 a	3.468 fg
Sabir-beg × -18˚C × Four weeks	115.833 a	102.333 f	145.000 d	291.667 jk	24.733 jklm	31.203 ab	0.243 ijkl	15.635 a	3.801 de

Conclusion

The wheat varieties had a highly significant variance for all the studied traits. Sabir-beg had the highest and most desired performance for most of the traits including grain yield, while Hereford had the highest spike number per unit area, and Skalnige showed the highest grain number/spike. The incubation temperatures had a highly significant effect on most of the traits including grain yield. Days to flowering was reduced with -18°C incubation. The same pattern was also indicated for days to maturity, spending less days to maturity when vernalized at -18°C treatment.

Period of incubation for different temperatures had a significant effect on plant height, days to maturity, and 1000 kernel weight. This result indicates the satisfaction period of two weeks incubation at low temperature for the wheat varieties. Sabir-beg is a local variety that is mostly adapted to the local environment, requiring less vernalization, as the interaction effect of this variety at low incubation temperature had less effect on the studied traits.

The interaction of varieties×incubation temperature had a highly significant effect on all traits except biomass yield. The interaction of 4°C with different wheat varieties had superiority to direct some of the important traits. It also seems that grain number is mainly genotype-related as the incubation treatment has no significant effect on the improvement of this trait. This result assured the necessity of involving the European varieties in field trials to be used directly or in a breeding program to improve the grain number/spike as an important trait related to yield. The high number of 40.566 grain/spike for Skalnige variety compared to 22.633 grain/spike of local variety is a big support for the importance of involving the European variety in the improvement program of wheat in the Kurdistan region of Iraq. The varieties persistency is also an outcome of their interaction with the incubation period, as the incubation of four weeks period had no recognized change on the traits compared to the two weeks incubation. The interaction effect of 4°C×four weeks period had allowed the flowering to be advanced, allowing the plant to stay longer in the flowering and maturation period giving a higher number of spike per unit area, which indicates the effect of low incubation temperature on the varieties under local environmental conditions.

Both interactions of varieties×incubation temperature and varieties×incubation period had a similar effect on the traits studied, as harvest index, biomass yield, and grain yield were highly significant for both interactions, but with less effect on improving the yield. This could refer to the availability of different alleles for VRNI gene, reducing the necessity of vernalization period or reversing its effect. Cold treatment (vernalization) can not alone regulate the flowering initiation, coordinating the responsive interaction of the plant with its environment, it is also necessary to express floral activation. The interaction of all three factors showed a highly significant effect on all the traits. Cultivation of the European varieties under study under local environmental conditions is recommended to boost the increase of grain yield in wheat. *In vitro* vernalization for the grains is a substitute solution for completing the required vernalization period, that might be raised by delaying sowing or unsatisfied cold period during planting and growing season to vernalize such varieties. Further filed trial is suggested in the future, involving more variable wheat genotypes to evaluate the yield resiliency and homeostasis of broad and diverse alleles under local environmental conditions.

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