FROST HARDINESS OF IRAQI WHEAT GENOTYPES.

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ABSTRACT

Frost hardiness level in winter cereals is especially temperature dependent. The low temperature which kills 50% of plants (LT_{50}) is considered to be a standard indicator of frost hardiness level of plant. Frost hardiness is considered the most important parameter for the field survival which is the ultimate measure of winter-hardiness of a cultivar. This study aimed to determine the genetic level of frost hardiness of five Middle Eastern varieties (Abu-Ghraib, Fatah, IPA 95, IPA 99, and Sham 6) in comparison to the European cultivar (Claire). All of the Middle Eastern varieties tested showed very similar LT_{50}'sin the non-acclimated state, whilst when of acclimated they responded in different ways to freezing temperatures. Abu-Ghraib and Claire showed more tolerance than the other cultivars.

Keywords: LT_{50}, Iraqi wheat genotypes, European winter wheat, Frosthardiness.

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INTRODUCTION

The ability of any plant species to withstand low temperature is one of the primary factors determining its adaptation area and distribution. Spring growth habit wheat types avoid many of the limitations imposed by enforced by winter conditions. However, winter growth habit types must live on these extreme environmental conditions. The survival is achieved through a process of cold acclimation leading to increased hardiness (Levitt, 1972). Frost hardiness level in winter cereals is especially temperature dependent (Fowler and Carles, 1979) and the difference in cold hardiness potentials among cultivars were apparent from the early stages of acclimation. Winter wheat growth and production are frequently affected by inconsistent winter survival. Skinner and Garland-

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Campbell (2008) claimed that the ability of wheat plants to survive the frozen state for extended periods of time is crucial to stay alive during the hard winter months in Canada and the northern US. The most important thing for field survival which is the ultimate measure of winter-hardiness of a cultivar (Skinner and Garland Campbell, 2008). However, field survival is not fit as a tool in selection because its unreliable because of the variable winter severity with differential winter kill (Gusta *et al.,* 2001; Skinner and Garland Campbell, 2008), and microgeographic environmental variation resulting in the plants throughout a field being exposed to a wide variation in temperature stress (Fowler and Carles, 1979). Typically the genetic level of frost hardiness of wheat cultivars is measured as LT_{50} the temperature which kills 50% of plants under test. The 50% killing point is the most readily measured and has now has become standard, which should be determined under controlled conditions (Levitt, 1980). This study aimed at determining the genetic level of frost hardiness of Middle Eastern varieties brought to the UK from Iraq compared with a European winter wheat cultivar (cv. Claire). In addition to evaluate differences in frost resistance among cultivars, therefore this will provide basic information for the future studies on these cultivars.

MATERIALS AND METHODS

In order to monitor the frost tolerance ability to six varieties were used, five Iraqi wheats:

1- Abu-Ghraib……………..winter wheat
2- IPA 99……………………spring wheat
3- IPA 95……………………spring wheat
4- Sham 6…………………...spring wheat
5- Fatah……………………..spring wheat.

In addition,one European wheat

6- Claire....................winter wheat

The experiment was carried out at Plymouth University during July 2009. The wheat cultivars were established in plastic trays (37 x 23 x 5 cm). Each tray contained three rows (12 plant each row) with two trays representing one replicate. The plants were raised outside during July and August when light levels where high and mean temperatures were optimal for growth. A Complete Randomised design was used with three replicates. This number of plants was duplicated in order to attain two groups for each cultivar, one for acclimation (two weeks at <4 °C in cold store), and the other one for non-acclimation. The cold store was programmed at 8h photoperiod with light intensity 7.6 $\mu mol.m^{-2}.sec^{-1}$ less than 4 º C. The Target temperature of the cold store was not achieved but was 1.5 ºC with an oscillation of +/- 1.0 ºC. The acclimated group was sown two weeks before the non-acclimated one to be ensure that all plants should place into frost chamber at the same growth stage (decimal growth stage GS $Z_{1.4}$& $Z_{2.2}$) when plants had established 3-4 leaves.

The total treatment design was as follows:

6 varieties x 2 acclimation treatments x 6 freezing temperatures x 3 replication

A freezing chamber (SANYO M533 incubator) was programmed to the following regime: 0, -2, -4, -6, -8, -10 °C with atwo hour held at each test temperature. The plants were removed at the end of the two hours hold at each temperature and immediately placed at 4 ºC to defrost overnight. Finally, all trays were transferred to the field to recover for two weeks prior to damage score being collected(Table 1).

Table 1: *The damage scores after 14 days recovery* (Fuller *et al.*, 2007).

Score	Description
0	Whole seedling un-damaged
1	Less than or equal 50% of seedling damaged
2	More than 50% of seedling is damage
3	Seedling is completely dead.

The data were analysed as a balanced ANOVA using Minitab v.15. LT_{50} was determined by applying the logistic differential equation for curve fitting using sigma plot:

$$S\% = 100/(1 + e^{a-rt}).$$

Where S: is the survival percentage, a and r: constants, and t: the temperature (Robert, 1974).

RESULTS AND DISSCUSSION

Data showing the frost damage to seedling wheat varietiesafter exposure to various temperatures are presented in Table 2 which showed that plants were increasingly damaged as the temperature was lowered to -10 ºC. Analysis of variance for the data (Tables 2&3) indicated that there is a significant difference in the damage of varieties. All of the Middle Eastern varieties were damaged approximately to the same degree except Abu-Ghraib which was similar to the European cultivar Claire. All varieties demonstrated a tendency to become cold acclimated when they were incubated at 4 °C. Significantly, the non-acclimated plants were damaged at temperatures higher than the acclimated plants. Whilst there is no clear effect of the interaction between the variety and temperature on the damage scores, the analysis showed a significant effect of the interaction between the genotypes and acclimation treatments. However, acclimation and temperature were correlated in their effect on seedling damage. The acclimated plants tolerated temperatures much lower than non-acclimated plants. There was no significant three-way interaction between factors.

Table 2: *The average of score damage to wheat cultivars in acclimation and non-acclimatio after exposure to negative temperatures.*

Varieties	Abu-Ghraib		Fatah		IPA 95		IPA 99		Sham 6		Claire	
Temperature °C	**NA***	**CA****	**NA**	**CA**	**NA**	**CA**	**NA**	**CA**	**NA**	**CA**	**NA**	**CA**
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-4	0.5	0.2	0.6	0.4	0.5	0.3	0.6	0.3	0.6	0.3	0.3	0.2
-6	2.0	0.7	1.8	1.0	1.6	1.1	1.6	0.9	1.6	1.1	1.2	1.1
-8	2.6	1.2	2.6	1.4	2.9	1.7	3.0	1.5	2.9	1.5	2.5	1.3
-10	3.0	2.9	3.0	2.9	3.0	3.0	3.0	2.9	3.0	2.9	3.0	2.9
Mean	1.3	0.8	1.3	0.9	1.3	1.0	1.4	0.9	1.3	1.0	1.2	0.9
Mean V	1.1		1.1		1.2		1.2		1.1		1.0	

*Non-Acclimated plants ** Cold Acclimated plants

Table 3: *L.S.D values of study factors.*

Variables	**P value**	**L.S.D $_{(0.05)}$**
Varieties	0.027*	0.101
Temperature	0.000***	0.101
Acclimation	0.000***	0.058
Varieties × Temperature	0.787	N.S
Varieties × Acclimation	0.067*	0.143
Temperature × Acclimation	0.000***	0.143
Varieties × Temperature × Acclimation	0.422	N.S

*Significant. **Very significant.*** Highly significant. N.S: non-significant.

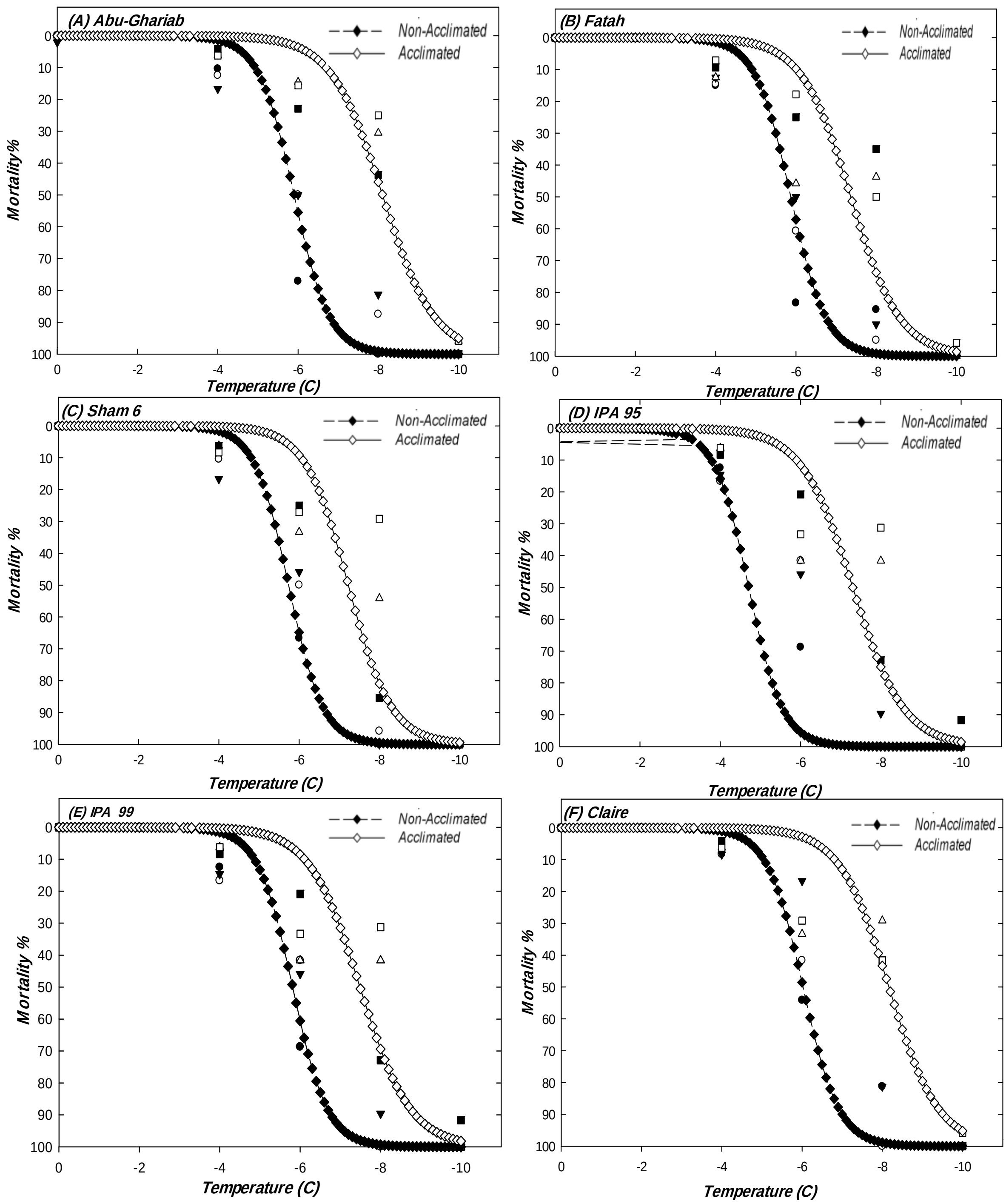

Figure 1: *The logistic differential curves of cultivars Mortality after exposure to freezing temperatures.*

The genetic ability of cultivars to withstand cold temperatures is the most important information that is required from this experiment. LT_{50} (50% killing point) was determined from the logistic differential curves of the seedling mortality after two hours exposure to different negative temperatures (Figure 2 A, B, C, D, E, F and Table 4). It was very clear that the non-acclimated plants had a LT_{50} approximately at the same temperature but the acclimated plants showed different responses. Genotypes were different in their LT_{50} with Abu-Ghraib and Claire cultivars being similar and significantly showing LT_{50} temperatures substantially lower than the other cultivars.

Table 2:*The LT50s for the varieties in acclimated and non-acclimatedstate.*

cultivars	Type and origin	LT_{50} Un-cclimated	LT_{50} Acclimated	LT50 in literature
Claire	WW European	-5.926 ºC	-8.065 ºC	LT50 for cv.º Claire are -5.95 C and -9.51 ºC in NA and CA state respectively (Fuller et al., 2007), but for Middle Eastern varieties there is no LT50 in the literature.
Abu-Ghraib	WW Middle Eastern	-5.803 ºC	-8.005 ºC	
Fatah	SW Middle Eastern	-5.774 ºC	-7.266 ºC	
Sham 6	SW Middle Eastern	-5.640 ºC	-7.127 ºC	
IPA 95	SW Middle Eastern	-4.608 ºC	-7.199 ºC	
IPA 99	SW Middle Eastern	-5.713 ºC	-7.386 ºC	

Wheat cultivars vary in their ability to survive low temperature and small differences in genetic potential can lead to large variability in low temperature tolerance (Fowler *et al.,* 1999). The ability of wheat cultivars to tolerate low temperature stress is determined by complex physical and biochemical interactions that are dependent upon genotypic and environmental factors. The level of cold hardiness of wheat cultivars can be measured as LT_{50} and in this experiment, this statistic was shown to be useful to distinguish between the cultivars.

Differences in cold hardiness among the wheat cultivars were apparent from this experiment. For the non-acclimated plants, all cultivars, even the European cultivar (Claire), behaved similarly with small differences in their LT_{50} varying from only -4.6 to -5.9. This actually demonstrates that even in an un-acclimated state, wheat does demonstrate constitutive frost tolerance down to about -5 to -6 ºC. When the cultivars were acclimated, all showed increased frost tolerance butresponse to cold acclimation (CA) differed between the cultivars. The Iraqi cultivars with the exception of Abu-Ghraib typically only showed a 1 to 2 ºC improvement in LT50 and there were only small cultivar to cultivar differences. Abu-Ghraib however responded to cold acclimation better than the rest of the cultivars and was much more similar to the European winter wheat cultivar Claire. Both of these cultivars are classified as winter wheats and they are therefore expected to have a genetically greater ability to acclimate. As shown in Table (4) the LT_{50} for Claire in the non-acclimated state was -5.926 ºC which was almost identical to that previously quoted by Fuller *et al.* (2007) (-5.950 ºC), but it differed by1.445 ºC difference in LT_{50} when acclimated. The reasons for this difference are unclear but may reflect slightly different acclimation treatments.

Despite all genotypes under study responded to acclimation process, the responses were different among the genotypes. The acclimation improved the LT_{50} by 0.493 ºC in Sham 6 while the improvement was 2.591 ºC in IPA 99and the other wheat cultivars ranged between these two. Generally, there is no information in the literature regarding the frost hardiness levels of Iraqi wheat cultivars so this is new information. It is clear that the classified Iraqi spring wheats were less able to acclimate than the winter wheat demonstrating the loss of frost hardiness associated with the spring habit as described (Christine and Law, 1979). This is attributed to an associated negative selection when selecting for low vernalisation characteristic of the spring wheats. As has been shown (Sutka *et al.*, 1999) frost tolerance QTL's are closely linked to the VRN genes on chromosome 5 and this linkage is difficult to break.

It is clear from the results that all cultivars had an LT_{50} in the non-acclimated state around -5 ºC. This temperature is considered as inherent constitutive moderate frost tolerance(Fuller *et al.*, 2007). Matthew (2005) mentioned that the herbaceous plants from temperate regions can tolerate temperature ranging from -5 ºC to -30 ºC depending on the species i.e. rye plant grown at non-acclimating temperatures (~22 ºC) are killed by -5 ºC but however when it has been acclimated it is able to withstand temperature down to -30 ºC. Within wheat there is variation in frost tolerance with LT50's from -5 to -25 °C with the more resistant varieties selected for severe winter climate zones in continental America and continental Europe. Maritime wheat varieties require less frost tolerance and typically, UK wheats only show moderate frost tolerance as demonstrated here by the cultivar Claire.

Frost resistant cultivars reportedly harden faster and deharden more slowly than the susceptible genotypes because the acclimation processes have different threshold induction temperatures differing them in cold tolerance (Gusta *et al.*, 1979). It was not possible to test this in the current experiment. There is also potentially a difference among cultivars regarding the threshold temperatures (above 0 ºC) at which acclimation is initiated and threshold induction temperatures for cold acclimation in wheat, barley and rye have been reported to be positively correlated with frost tolerance between and within species (Fowler, 2008). The genotypes under study here might have varied in their frost tolerance according to differences in threshold induction temperatures for acclimation but it is anticipated that the temperature used, +1.5 ºC, should have up-regulated acclimation in all cultivars. Acclimation has also been reported to proceed in two stages in cereals, depending on the sequential action of chilling (> 0 ºC) and sub-lethal freezing (-1 to -5 ºC) temperatures. Cold acclimation requires energy to be maintained and with seedlings this energy is supplied by seed reserves or by photosynthesis (Fowler and Limin, 2007; Limin and Fowler, 1985). The development and maintenance of available carbohydrates supply with retarded vegetative growth is essential for cold temperature acclimation of wheat plants to occur efficiently. This observation had led to the suggestion that acclimated and non-acclimated cultivars with high growth activity at low temperature might be reflecting an inability to acclimate in autumn. Under controlled experimental conditions, it is necessary that sufficient light for photosynthesis is provided during acclimation (Gabriella *et al.*, 2009) and the levels used in this experiment were within a range that can stimulate photosynthesis during acclimation.

It can be concluded from the finding of this study, that all Middle Eastern varieties tested showed very similar LT_{50}'s in the non-acclimated state and this demonstrated that they have an innate ability to survive freezing temperatures down to approximately -4 to -5 ºC similar to European and North American wheats. Following acclimation, all varieties showed significant improvement in their response to freezing but the degree of response varied among the cultivars. Abu-Ghraib and Claire both showed the best response to acclimation commensurate with their winter wheat status whilst the remaining cultivars were much more like spring wheats.

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التقسية ضد الصقيع لأصناف الحنطة العراقية .

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المستخلص

التقسية للصقيع (Frost hardiness) تعتبر من اهم المعايير الحقلية للحنطة لتبقى حية خلال الشتاء وهو والمقياس النهائي لأي صنف ليجتاز درجات الحرارة المنخفضة خلال الشتاء. تهدف هذه الدراسة الى تحديد المستوى الوراثي للتقسية ضد الصقيع لخمسة اصناف عراقية (أبو غريب، فتح، اباء95، اباء99، وشام6) ومقارنتها مع الصنف الاوربي المتحمل للصقيع (كلير Claire)، باستعمال درجة الحرارة التي من الممكن ان تقتل 50% من النباتات (LT_{50}) والتي تعتبر المؤشر القياسي لمستوى التقسيه لاي نبات. جميع الاصناف تحت الدراسة اعطت تقريبا قيمة متشابهة للـ (LT_{50}) في حالة النباتات غير المؤقلمة مبينة القابلية الوراثية للحنطة لتحمل درجات الحرارة المنخفضة من غير اقلمتها الى تحمل درجة الحرارة المنخفضة. عندما عرضت الاصناف الى عملية الاقلمة، أستجابت بشكل متفاوت لمعاملات الحرارة, حيث بينت النتائج ان صنف ابوغريب اعطى اعلى قابلية لتحمل درجات الحرارة المنخفضة مقارنة مع بقية الاصناف مؤكدا انه حنطة شتوية، اما الاصناف البقية فتميل الى ان تكون اصناف ربيعية. تعتبر هذه الدراسة اساس لدراسات اخرى على الاصناف العراقية كما وتعبر الاولى من نوعها على هذه الأصناف.

الكلمات المفتاحية : LT_{50} ، أصناف الحنطة العراقية ، الحنطة الأوربية الشتوية ، التقسية ضد الصقيع .