International Journal of Food, Agriculture and Veterinary Sciences ISSN: 2277-209X (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jfav.htm
2015 Vol. 5 (3) September-December, pp. 43-54/Malakshaha
Research Article

AMYLASE ACTIVITY AND YIELD IN SPRING WHEAT CULTIVARS (*Tritium aestivum L.*) DURING PRE-HARVEST SPROUTING IN NORTHERN IRAN

***Ahmad Ahmadpour Malakshaha**
Department of Environmental Sciences Savitribai Phule Pune University, Pune, 411007
**Author for Correspondence*

ABSTRACT
Pre-harvest sprouting (PHS) refers to germination of seeds in physiologically mature spikes prior to harvest. In northern part of Iran (Gorgan and Mazandaran), it is very common and occur three or four times per ten years. The highest damage due to PHS of wheat in north of Iran was about 22000 ha, causing severe economic losses and reduction in grain yield quality, test weight, grain functionality and seed viability. Sprouting in wheat induces the synthesis of enzymes like α, ß and total amylase, which influences grain yield and bread making quality. PHS also negatively affects starch and proteins. The results revealed that selected genotypes of spring wheat significantly differed on the basis of starch, proteins and activities of α, ß and total amylase, percentage and severity of PHS and yield during MI. After 21 days of MI starch and protein contents as well as yield was reduced highly. However, activities of total, α and ß-amylase, percentage of PHS and severity were increased as compared to 7 and 14 days of MI. Starch and protein had shown positive correlation while total, α and ß-amylase activity, percentage of PHS and severityhad shown negative correlation with grain yield after 21 days MI. MI. Starch, protein and duration MI showed negative direct effect, while the negative indirect effect was observed on grain yield due to total amylase activities. Regression analysis shown for starch, proline and yield were decreased but total amylase activity was increased after 7, 14 and 21 days of MI.

Keywords: *PHS, Starch, Protein, Amylase, Yield, Wheat*

INTRODUCTION
At present wheat crop is exposed to several biotic and abiotic factors like PHS, drought, salinity that contribute to losses in yield, grain quality and economic grain (Bi *et al.*, 2014). Pre-harvest sprouting (PHS) in wheat is a serious problem in the regions of the world where the rainy season tends to overlap with the harvest season (Gerjets *et al.*, 2010; Nakamura *et al.*, 2011).

The α-amylase widely exists and participates in many physiology processes in plants and hydrolyze starch to sugars.

The expression of α-amylase affects the germination rate, cold tolerance and production of seed (Masojc and Milczarski, 2009).

The relationship between α-amylase activity and PHS resistance very remarkable. The activity of α-amylase increases quickly once the seed absorbs enough water and then promote the seed sprouting (Wang *et al.*, 2008).

The activity of α-amylase was significant different between the PHS resistant and sensitive varieties in wheat (Wang *et al.*, 2008; Gao *et al.*, 2013).

Many spring wheat varieties are susceptible to pre-harvest sprouting (Biddulph *et al.*, 2008). PHS susceptible cereal varieties typically lack adequate levels of seed dormancy to avoid early sprouting during wet harvest periods.

It is generally induced during kernel development and depends on environmental conditions as well as on physiological, morphological and genetic properties of cereals.

The understanding of physiological, morphological, enzymological and genetic factors during PHS is yet obscure.

Damage caused by PHS has often been associated with increased levels of α, amylase, ß and total amylase activities in the kernel. High α-amylase activity levels negatively affect the nutritional and end-use quality of grains (Mares and Mrva, 2008).

Several tests assessing this damage due to PHS are based on α-amylase activity levels at harvest, contents of starch and proteins, morphology of spike and seed coat colour. Up till now very scanty work is available on the relationship between the PHS and α-amylase activity in wheat and barley (Lin *et al.*, 2008).

The α-amylase activity, starch, sugars, carbohydrates and protein contents are the key factors involved in PHS of wheat genotypes.

The level of α-amylase activity depends on the genotypes and environmental conditions as well as genotypes ×environment. In many wheat and rye genotypes, α-amylase activity remains low until harvest ripeness, whereas it may increase to excessive levels at harvest maturity in certain genotypes of triticale and wheat (Biddulph *et al.*, 2008; Gao and Ayele, 2012).

The aim of present study was to find out the correlation between levels of α, ß and total amylase activity along with starch and protein content in selected PHS tolerant and sensitive genotypes of wheat and its impact on grain.

MATERIALS AND METHODS

The field experiment was conducted using completely randomized block design with three replications in Autumn season in the year (2008-2012) at Agriculture Research Station, Baye Kola, using selected genotypes such as Nai60, N-80-19, N-87-12, N-86-12 and N-87-8 under different conditions of mist irrigation (7, 14 and 21 days). The analysis of starch (Thayumanavan and Sadasivam, 1984), protein (Lowry *et al.*, 1951) and α, ß and total amylase (Sadasivam and Manikum, 1996) activity was attempted by using standard protocols. The data obtained was statistically analyzed using SPSS software (version 16).

Model Fitting

The relationships between studied traits and MI were evaluated by fitting linear and non-linear regression models by SAS software. In this study a segmented model was applied as non-linear model which following as:

$Y = a + bx$ if $x<x0$ [1]

$Y = a + bx0$ if $x \geq x0$ [2]

where Y is the studied physiological parameters, a is intercept, b is the rate of increase or decrease in studied traits, $x0$ is turning point between two phases and x is mist irrigation duration. The internal validity of the models was tested by coefficient of determination (R2).

RESULTS AND DISCUSSION

Effect of PHS on Physiological and Biochemical Parameters

The results regarding mean comparison of effects of PHS on physiological and biochemical parameters in wheat genotypes presented in Table 1, revealed that by contrast, all the PHS sensitive genotypes in third step of MI showed lowest contents of starch, protein and highly reduced yield as compared to first and sconed MI.

However, other variables in third step of MI, like total amylase, α-amylase and ß-amylase were higher than other two MI (first and sconed).

The results also indicated that the contents of starch (19), protein (22.23) and yield (0.04) in highly PHS sensitive genotypes such as N-87-8 were significantly decreased during third step of MI. While total amylase (66.5), ß-amylase (34.25) and α-amylase activities (32.25) were highly stimulated during third step of MI. But the parameters such as starch (23), protein (30.26) and yield (0.5)in PHS tolerant genotypes such as N-86-12 were not negatively affected.

International Journal of Food, Agriculture and Veterinary Sciences ISSN: 2277-209X (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jfav.htm
2015 Vol. 5 (3) September-December, pp. 43-54/Malakshaha
Research Article

Table 1: Mean Comparison of Effects of PHS on Physiological Parameters in Wheat Genotypes Under Different MI

Parameters MI	Genotypes	Starch /mg/g	ß-amylase /mg/g	α-amylase / mg/g	Protein / mg/g	Total amylases /mg/g	Yield kgm^2
7	Nai60	140.0 a	2.300f	1.440e	100.0 a	3.440 h	0.8070a
	N-80-19	182.0 b	2.660 f	1.440e	95.90a	3.660 h	0.9070a
	N-87-12	140.0 b c d	3.990 f	5.540 cde	84.50ab	8.540fg h	0.8630a
	N-86-12	154.0 b c	2.660 f	2.990 de	98.20a	4.990g h	0.8030a
	N-87-8	135.00 c d e	2.440 f	3.660 de	79.30ab	5.660g h	0.8570a
14	Nai60	44.00 de	12.72d e	2.690 de	58.97bcd	14.41e f	0.8000a
	N-80-19	34.00 c d e	18.67b	16.630c d	48.98bcd	35.30c d	0.2520c
	N-87-12	32.00 c d e	18.15 b	16.63b	36.26cd	34.78 b	0.1600cd
	N-86-12	52.00 cd e	5.49 f	3.990cde	77.22abc	8.150 h	0.7200a
	N-87-8	38.00 d e	24.10c d	23.44b	37.15cd	47.54 d	0.1900cd
21	Nai60	37.0 de	17.36 c d	7.130de	38.94d	20.50 e	0.1500cd
	N-80-19	33.0 de	22.06b	20.540 c	28.59d	42.61b c	0.1070cd
	N-87-12	15.0 e	22.86 a	20.72 a	19.74 d	42.58 a	0.06000cd
	N-86-12	23.0 e	8.920 e f	6.150 e	30.26bcd	14.98 f g	0.5000b
	N-87-8	19.0 e	34.25 c	32.25 ab	22.23d	66.50 b c	0.0400d

Relationship Regression between MI X Starch in Different Genotypes

Starch ranged from 15 to 182 mg gr across genotypes (table 1). A segmented model was fitted for describe relationship between starch and days after MI for all genotypes. The results indicated that genotypes were approximately similar in terms of parameters. Therefore, after starting of MI the starch values reduced linearly (-16 mg gr) with day to about 15 days and then the values of starch were constant to 21 days after MI. There is an exception only for N-80-19 genotype, which starch value was mostly reduced from other genotypes (-21 mg gr per day) (Table 2; Figure 1). The tolerant variety showed more accumulation of starch as compared to PHS sensitive genotypes of wheat.

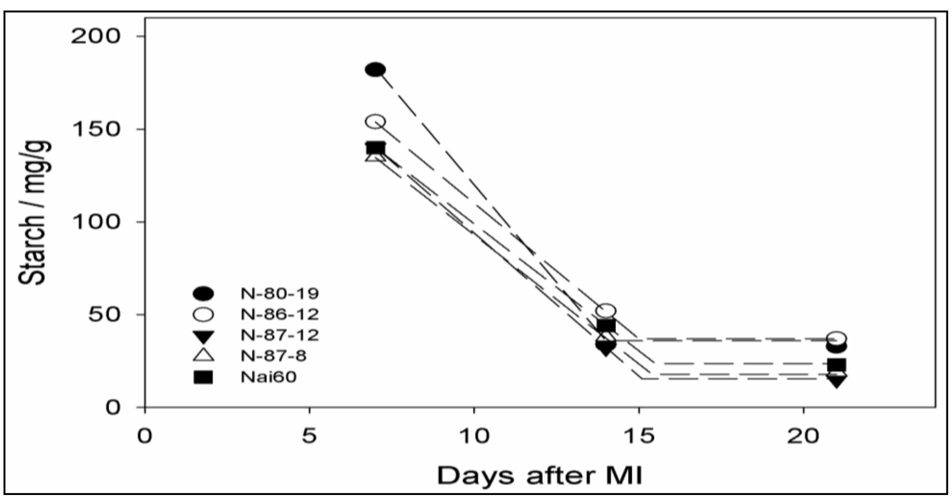

Figure 1: Linear Regression between MI × Starch In Five Genotypes Of Wheat

International Journal of Food, Agriculture and Veterinary Sciences ISSN: 2277-209X (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jfav.htm
2015 Vol. 5 (3) September-December, pp. 43-54/Malakshaha
Research Article

Table 2: Linear Regression between MI X Starch In Different Genotypes Of Wheat

Traits	Genotypes	a	b	X0	R^2
Starch	N-80-19	330	-21	14	0.98
	N-86-12	256	-14.6	15	0.99
	N-87-12	2.48	-15.4	15.1	0.98
	N-87-8	232	-13.19	15.4	0.98
	Nai60	236	-13.7	15.5	0.99

a=Intercept b=Slope X0= In depended variable

Relationship Regression between MI× Total Amylase in Different Genotypes

Total amylase ranged from 66.5 to 3.44mg/g across genotypes Table 2. Regression analysis shown for all genotypes N-80-19, N-86-12, N-87-12 and Nai60 using the linear model were increased after 6, 14 and 21 days (2.7821, 0.7136, 2.4314 and 1.2186 respectively). There was significant level between MI in terms of values of total amylase based on regression analysis. Total amylase values in the N-87-8 was increased with increased MI which it may be indicated increasing of sensitive this genotype than other genotypes (Table 4; Figure 2).

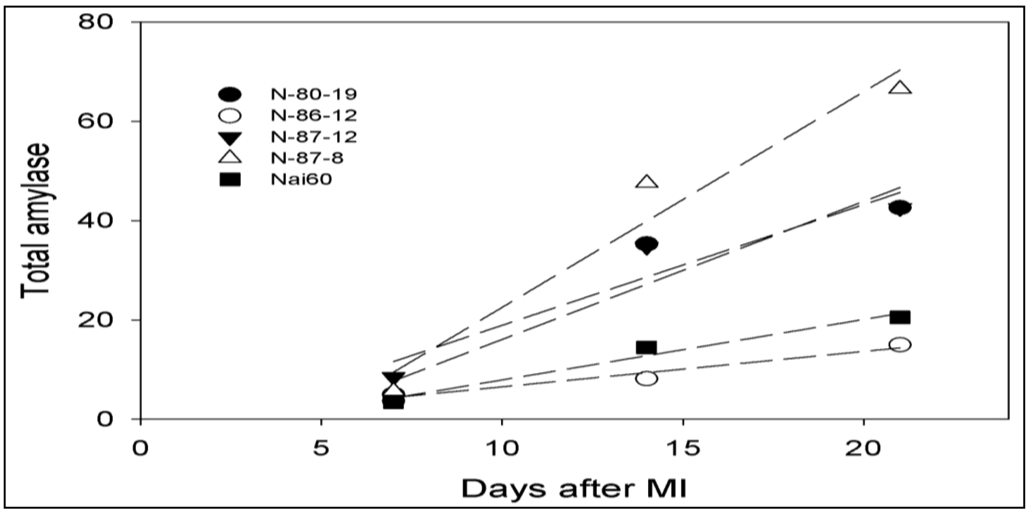

Figure 2: Linear Regression between MI X Total Amylase in Different Genotypes of Wheat

Table 3: Linear Regression between MI X Total Amylase in Different Genotypes of Wheat

Traits	Genotypes	a	b	X0	R^2
Total amylase	N-80-19	-11.76	2.78		0.88
	N-86-12	-0.61	0.71		0.96
	N-87-12	-5.40	2.43		0.91
	N-87-8	-20.94	4.34		0.95
	Nai60	-4.27	1.21		0.97

a=Intercept b=Slope X0= in depended variable

Relationship Regression between MI× Protein and In Different Genotypes

Protein ranged from 100 to 38.94 mg gr across genotypes Table 2. The results of segmented model indicated that there is relationship between protein and days after MI for all genotypes and wheat genotypes were approximately similar in terms of parameters (table 4.21 & Figure 4.25). Therefore, estimated reducing slope for all genotypes N-80-19, N-86-12, N-87-12, N-87-8 and Nai60 using the segmented model were decreased linearly with day to -14.8079, -4.8529, -4.6257, -4.0764 and -4.3614 respectively (Table 5; Figure 3).

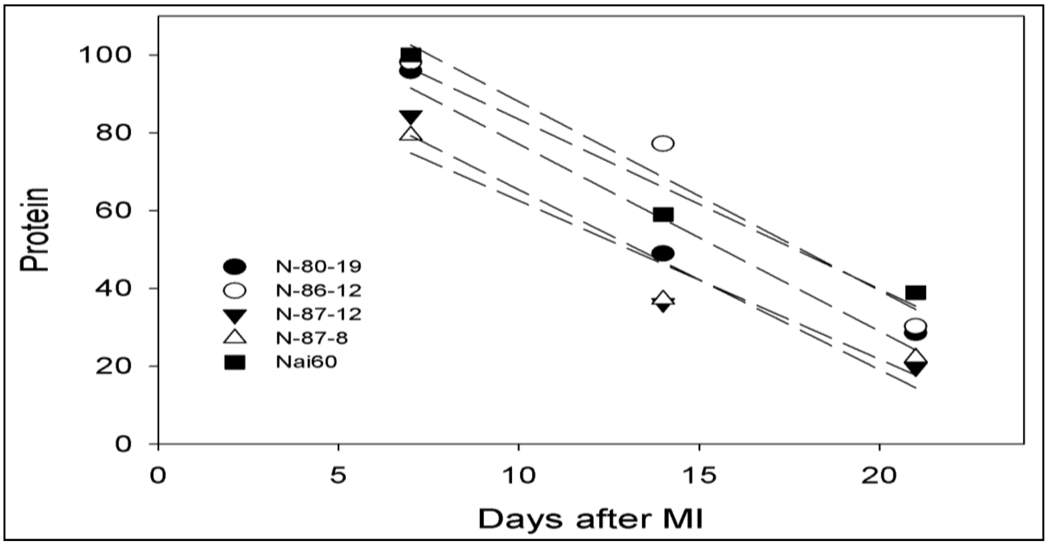

Figure 3: Linear Regression between MI X Protein in Different Genotypes of Wheat

Table 4: Linear Regression between MI X Protein in Different Genotypes of Wheat

Traits	Genotypes	a	b	X0	R^2
Protein	N-80-19	125.13	-4.80		0.95
	N-86-12	135	-4.85		0.95
	N-87-12	111.59	-4.62		0.93
	N-87-8	103.29	-4.07		0.93
	Nai60	127.03	-4.36		0.96

a=Intercept b=Slope X0= In depended variable

Relationship Regression between MI× Yield in Different Genotypes

Yield ranged from 0.907 to 0.04 kg across genotypes Table 2. A segmented model was fitted for describe relationship between yield and days after MI for all genotypes. The results indicated that genotypesN-80-19, N-87-12, N-87-8 were approximately similar in terms of parameters values but then the values of yield was constant to 21 days after MI.

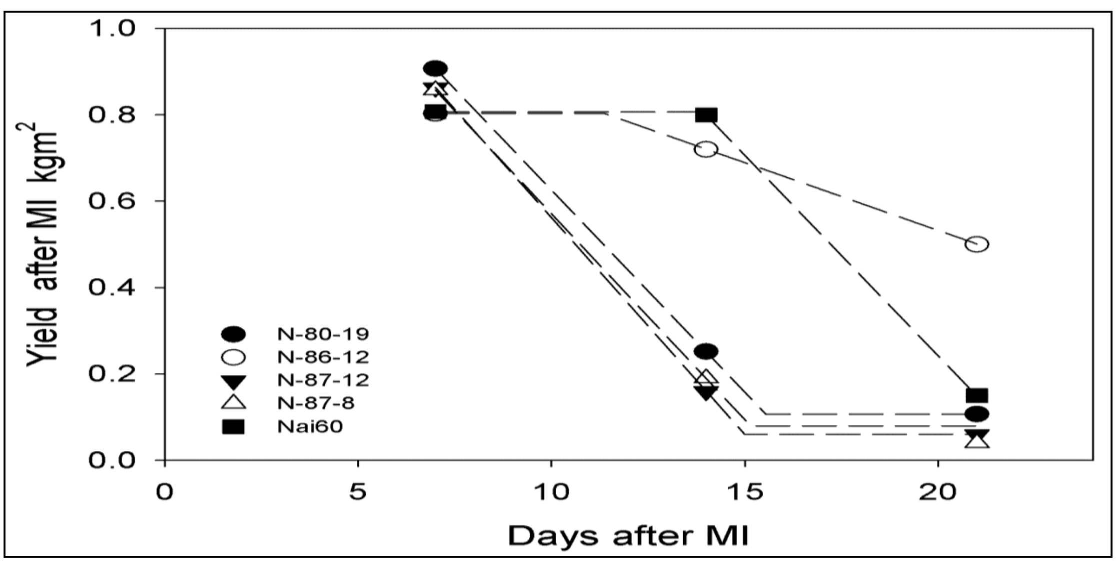

Figure 4: Linear Regression between MI X Yield in Different Genotypes of Wheat

International Journal of Food, Agriculture and Veterinary Sciences ISSN: 2277-209X (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jfav.htm
2015 Vol. 5 (3) September-December, pp. 43-54/Malakshaha
Research Article

The estimates for Nai60 using the segmented model were 13.92 days for turning point between the two phases (x_0) and -0.0929 kg for reducing slope (b) (namely, at days above x_0, yield values reduced linearly with day and before it values of yield was constant).
For N-86-12, estimates were 11.359 days and -0.0314 kg for x_0 and b and after that had procedure constant (0.5 kg). There were significant differences between genotypes in terms of values of yield based on regression analysis. Yield values in the N-86-12 were constant to 11.359 days after MI and with increased day which it may be indicated increasing of tolerance this genotype than other genotypes (Table 6; Figure 4).

Table 5: Linear Regression between MI X Yield in Different Genotypes of Wheat

Traits	Genotypes	a	b	X0	R^2
Yield	N-80-19	1.56	-0.09	15.54	0.99
	N-86-12	1.16	-0.03	11.35	0.98
	N-87-12	1.56	-0.10	14.99	0.99
	N-87-8	1.52	-0.09	15.15	0.98
	Nai60	2.1	-0.09	13.92	0.99

a=Intercept b=Slope X0= In depended variable

Simple Correlation (R) For the Selected Variables of Wheat Genotypes during PHS

The results of correlation between parameters such as starch (r=0.58*) and protein (r=0.91**) were positively correlated with grain yield after MI. The other parameters such as total amylase (0.90**), ß-amylase (0.88**), α-amylase (r=0.82**), percentage of PHS in spike (6.40**), severity of PHS in grain (0.97**) and duration of MI (r=0.82**) were negatively correlated with grain yield after MI (Table7).

Table 6: Simple Correlation (R) For the Physiological Traits of Wheat Genotypes under MI

Traits	Starch	Total amylases	ß-amylase	α-amylase	Protein	Percentage of PHS	Severity of PHS	Duration of MI	Yield
Starch	1								
Total amylases	-.510*	1							
ß-amylase	-.513*	.975**	1						
α-amylase	-.419*	.922**	.816**	1					
Protein	.765**	-.878**	-.868**	-.775**	1				
Percentage of PHS in pike	-.82**	-.55**	-.81*	-.85**	.77**	1			
Severity of PHS in grains	-.95**	-.66**	-.58**	-.81**	.80*	-.97**	1		
Duration MI	-.740**	.701**	.710**	.566*	-.888**	.74**	.70**	1	
Yield	.58*	-.90**	-.88**	-.82**	.91**	-6.40**	-.97**	-.82**	1

Path 8 Analysis for Direct and Indirect Effects on Selected Traits of Wheat Grains and Yield

Path analysis correlation revealed that starch, protein and duration MI showed negative direct effect (-2.0, -13.88 and -7.052) on grain yield respectively. The negative indirect effect on grain yield was observed due to total amylases (-16.618), ß-amylase (-11.667) and α-amylase (-12.654) (Table 8).

Table 7: Path Analysis For Direct And Indirect Effects On Selected Traits Of Wheat Genotypes

Variables	Path coefficient		Total correlation (Yield)
	Direct effect	Indirect effect	
Starch	-2.0	2.595	0.588
Total amylases	15.7	-16.618	-0.908
ß-amylase	10.58	-11.662	-0.885
α-amylase	6.206	-12.654	-0.829
Protein	-13.88	12.97	0.917
Duration of MI	-7.052	7.875	-0.828

Discussion

Starch Hydrolysis During of PHS

According to Dupont and Altenbach (2003) and Thitisaksakul *et al.,* (2012) starch is a major determinant of yield, accounting for 65-75% of the grain dry weight and up to 80% of the endosperm dry weight. Reductions in starch accumulation during PHS account or significant losses in grain yield (Tashiro and Wardlaw, 1989; Jenner, 1991; Hurkman *et al.,* 2003).

The results on starch content in selected elite lines of spring wheat indicated significant alterations with duration of MI (Table 2, 3; Figure 1). The tolerant variety showed more accumulation of starch as compared to PHS sensitive cultivar.

The grain starch is most important end product of cereals as they contain about 70% (w/w) starch (Thitisaksakul *et al.,* 2012; WHO, 2003). Studies on changes in starch content may help to improve and avoid its degradation during PHS (Shaik *et al.,* 2014).

The increase/decrease in starch content during germination is controlled by activity of α-amylase. In present investigation the wheat cultivar tolerant to PHS (N-86-12) showed less α-amylase activity and more starch in it. While opposite trend was observed in PHS sensitive variety (N-87-8). Our results on starch contents are in accordance with above findings.

Effects of PHS on Protein

The results shown in Table 2, 5; Figure 3 revealed that protein contents were reduced during second and third step of MI in PHS sensitive wheat genotype (N-87-8) as compared to PHST genotype (N-86-12). Protein metabolism during seed germination is highly important, which is degraded by enzyme protease, releasing different amino acids, which are utilized by developing embryo (Bewly and Black, 1994). The breakdown of protein is very fast in wheat grains showing sprouting under MI in PHS sensitive genotype. But in PHS tolerant genotype due to dormancy inducing compounds like phenols there is no seed germination and no utilization of reserve food material like protein and hence the PHST genotype showed high protein content.

The alterations in protein metabolism may act as biochemical marker for screening the PHST/ sensitiveness of wheat genotypes in breeding program. Analysis of protein accumulation/ degradation may serve as a reliable physiological indicator to screen the tolerance/ sensitiveness of wheat genotypes to PHS. Total protein contents are significantly affected by PHS, it appears that protein percentage is less sensitive to high temperatures (De Laethauwer *et al.,* 2013). PHS had a significant effect on protein percentage (Zhang *et al.,* 2014). Grain buyers use protein percentage as the surrogate measure for malting quality, due to the inverse relationship with starch content and positive correlation with diastatic power (Singh *et al.,* 2014).

Many researches like Awole *et al.*, (2012); Morris *et al.*, (2013); Oszvald *et al.*, (2014); Ade-Omowaye *et al.*, (2008); Fu *et al.*, (2014); Shewry *et al.*, (2010-2012); Shiferaw *et al.*, (2013) had indicated importance of protein in grain quality which changes according to environmental conditions like PHS and genotype

Amylase Activity

The α-amylase widely exists and participates in hydrolysis of starch. The expression of α-amylase was involved in plant metabolism and could affect the germination rate and production of seed (Autio *et al.*, 2001; Masojc and Milczarski, 2009). This may be due to activity of α-amylase that would increase quickly once absorbed enough water and then promoted the seed sprouting (Wang *et al.*, 2008). The activity of α-amylase was also found to have a significant difference between the resistant and sensitive varieties to PHS in wheat (Wu *et al.*, 2002; Lin *et al.*, 2008).

The results shown in Table 2, 4; Fig. 2 clearly indicated that during third step of MI (21 days) the PHS susceptible genotypes had shown very high activities of α, ß and total amylase as compared to the PHST genotypes. The results of present investigation are in agreement with many researches like Singh *et al.*, (2010); Xing *et al.*, (2010); Jaiswal *et al.*, (2012); Clarke *et al.*, (2005); Singh *et al.*, (2014)and Ghanbari and Mir (2013). They pursued monitoring of α-amylase activity, an enzyme that is involved in PHS, both at transcriptional and post-transcriptional levels during kernel development. They further explained that damage caused by PHS has often been associated with increased levels of α-amylase activity in the kernel. By converting starch into soluble sugars, high α-amylase activity levels negatively affect the nutritional and end-use quality of grain (Mares and Mrva, 2008). Yan *et al.*, (2008) reported that the flour of sprouted grain has a lower falling number, because the active α-amylases degrade the starch, resulting in poor baking quality and severe limitations in end-use application Wheat.

Several tests assessing this damage due to PHS are based on α-amylase activity levels at harvest ripeness (Lin *et al.*, 2008). Although PHS is often the primary source of increased α-amylase activity, several other sources of α-amylase may obscure this weak relationship (Lunn *et al.*, 2001). The different levels of α-amylase activity have been detected in cereals like wheat, rye and triticale, they all show a typical pattern during kernel development (Laethauwer *et al.*, 2013; Gao *et al.*, 2013; Rentzsch *et al.*, 2012; Biddulph *et al.*, 2008). According to Wu *et al.*, (2002) and Gao *et al.*, (2013) the relationship between α-amylase activity and PHS resistance was deemed to be very remarkable (Wang *et al.*, 2008).

Singh *et al.*, (2014) stated that sprouting in wheat produces the enzyme α- amylase which leads to losses in yield and quality. DePauw *et al.*, (2012) detected a significant positive correlation between values for germination of threshed kernels and levels of α-amylase. Singh *et al.*, (2010) showed that PHS is initially recognized by an elevated level of starch hydrolytic enzyme activities that primarily originate from α-amylases. These enzymes catalyze breakdown of endosperm starch and thus provide the initial energy needed for seed germination (Xing *et al.*, 2010)

According to Jaiswal *et al.*, (2012) α-amylase is involved in germination and PHS tolerance. Ghanbari and Mir (2013) revealed that PHS negatively affect subsequent grain quality, seed viability, seedling vigor and milling and backing properties, reduction in grain quality is caused by conversion of starch to glucose (sugar) by the enzyme α-amylase. The enzyme α-amylase is synthesized in the aleurone layer and scutellum and released in the endosperm to decompose the starch into sugars available for germination (Lunn *et al.*, 2001). Several factors contribute to increased PHS tolerance, such as reduced level of α-amylase activity in grains, the presence of inhibitors of germination, reduced water absorption by the grains (Mares *et al.*, 2009; Jacobsen *et al.*, 2013; Kaplan and Guy, 2004). The results of present study on α-amylase activity in the selected wheat genotypes corroborate with above findings and confirm that the activity of α-amylase play a crucial role in selection of PHST genotypes of in cereals like wheat, barley and rye.

Effect of PHS on Grain Yield and End Use Quality

PHS in wheat greatly affects the grain yield in different parts of the world resulting in to substantial financial losses to farmers and food processors. It also decreases the grain value to the producers by impacting four different primary grade determinants grain quality and end use quality (Gao *et al.*, 2013; Masojc *et al.*, 2013; Jaiswal *et al.*, 2012; Himi *et al.*, 2012; DeLaethauwer *et al.*, 2013; DeLaethauwer *et* *al.*, 2012; Zhang *et al.*, 2014). Many researchers reported significant economic losses due to a reduction in grain yield during PHS in different crops including wheat and other cereals (Liu *et al.,* 2008; Singh *et al.*, 2014; Yang *et al.*, 2014; Kulwal *et al.,* 2012; DePauw *et al.,* 2012).

The results of present study are in close agreement with above findings. Grain yield was very low in PHS sensitive wheat genotypes of spring wheat as compared to tolerant genotypes. This may be due to degradation of starch by the elevated levels of amylase activity and high percentage of PHS and severity. Starch accounts for 64-74% of the total dry weight of wheat grains and hence if it is degraded it results into loss in grain weigh and yield (McCaig *et al.*, 2006; Kulwal *et al.*, 2012). The properties of starch are important for determining the end-use quality of wheat flour and its degradation lead to loss in end use quality of wheat. Degradation of native starch granules negatively affect quality of various products made from wheat flour. The primary reason for α- amylase accumulation in the grain is delayed harvest due to wet weather, causing breakdown of grain quality (DeLaethauwer *et al.*, 2012; Kondhare *et al.*, 2014). The source of elevated α-amylase activity is associated with pre-maturity sprouting and involves germination during early grain development when kernels are still at high moisture content (Lunn *et al.,* 2001; Shockravi *et al.,* 2012; Knox *et al.*, 2012). Rainfall at harvest, however, is the main cause of PHS inducing α-amylase activity (Wrigley, 2006). Even minor sprout damage can cause significant reductions in gluten strength of wheat flour making it unsuitable for bread making (Barbeau *et al.,* 2006; Knox *et al.*, 2012). The losses in grain yield, end use quality as well as grain quality during PHS in wheat grains depend on genotype, environmental conditions during grain development and interaction between these factors (De Laethauwer *et al.,* 2009), hence, cereal breeders constantly seek to improve tolerance to PHS in cereals (De Laethauwer *et al.*, 2012). In present investigation we have studied some few physiological, biochemical, and enzymological traits in spring wheat showing PHS in northern part of Iran. These markers may help the breeders in breeding program for selecting PHS tolerant varieties (Table 2, 6, 7, 8; Figure 4).

Conclusion

From the results of present study it can be concluded that the Iranian wheat varieties, which are late maturing are mostly sensitive to PHS, as rainfall occurs during harvesting period. The physiological attributes of grains such as starch and protein as well as activity of α, ß and total amylase may serve as the reliable physiological and enzymological indicators to identify the PHS tolerant or sensitive genotypes, under MI simulating the conditions of natural rainfall. The α, ß and total amylase activity play predominant role in PHS, which determines the loss in grain yield and end use quality.

REFERENCES

Ade-omowaye BIO, Akinwande BA, Bolarinwa IF and Adebiyi AO (2008). Evaluation of tiger nut (*Cyperus esculentus*) wheat composite flour and bread. *African Journal of Food Science* **2** 87-091.

Awole KD, Kettlewell PS, Hare MC and Agu RC *et al.*, (2012) Effect of environment and variety on the relationships of wheat grain physical and chemical characteristics with ethanol yield. *Journal of the Science of Food and Agriculture* **92** 77-5840.

Barbeau WE, Griffey CA and Yan ZH (2006). Evidence that minor sprout damage can lead to significant reductions in gluten strength of winter wheats. *Cereal Chemical* **83** 306-310.

Bewley JD and Black M (1994). *Seed Physiology of Development and Germination* (New York, Plenum Press) Book chapter.

Bhullar SS and Jenner CF (1985). Differential responses to high temperatures of starch and nitrogen accumulation in the grain of four cultivars of wheat. *Australian Journal of Plant Physiology* **12** 363- 375.

Bi HH, Sun YW, Xiao YG and Xia LQ (2014). Characterization of DFR allelic variation and their associations with pre-harvest sprouting resistance in a set of red-grained Chineses wheat germplasm. *Euphytica* **195**(2) 197-207.

Biddulph TB, Plummer JA, Setter TL and Mares DJ (2008) Seasonal conditions influence dormancy and pre-harvest sprouting tolerance of wheat (*Triticum aestivum* L.) in the field. *Field Crop Research* **107** 116-128. doi:10.1016/j.fcr.2008.01.003

Chrispeels MJ and Herman EM (2000). Endoplasmic reticulum-derived compartments function in storage and as mediators of vacuolar remodeling via a new type of organelle, precursor protease vesicles. *Plant Physiology* **123** 1227-1233.

Clarke FR, Clarke JM, DePauw RM and Fernandez MR *et al.*, (2005) Strategic approach to mitigating weather induced defects of wheat quality. *Euphytica* **143** 285-290.

De Laethauwer S, De Riek J, Stals I, Reheul D and Haesaert G (2013) α-Amylase gene expression during kernel development in relationto pre-harvest sprouting in wheat and triticale. *Acta Physiologiae Plantarum* **35** 2927-2938.

De Laethauwer S, Reheul D, De Riek J and Haesaert G (2009). The use of Vp1 in real time RT-PCR to select for pre-harvest sprouting tolerance in triticale. *Euphytica* **168** 379-384.

De Laethauwer S, Reheul D, De Riek J and Haesaert G (2012). Vp1 expression profiles during kernel development in six genotypes of wheat, triticale and rye. *Euphytica* **188** 61–70.

DePauw RM, Knox RE, Singh AK, Fox SL, Humphreys DG and Hucl P (2012). Developing standardized methods for breeding pre-harvest sprouting resistant wheat, challenges and successes in Canadian wheat. *Euphytica* **188** 7-14. DOI: 10.1007/s10681-011-0611-y

Derycke V, Haesaert G, Latre J and Struik PC (2002). Relation between laboratory sprouting resistance tests and field observations in triticale (x TriticosecaleWittmack) genotypes. In: *Proceedings of the 5th International Triticale Symposium, Poland* 123-133.

Dupont FM and Altenbach SB (2003) Molecular and biochemical impacts of environmental factors on wheat grain development and protein synthesis. *Journal of Cereal Science* **38** 133-146.

Fu BX, Hatcher DW and Schlichting L (2014) Effects of sprout damage on durum wheat milling and pasta processing quality. *Canadian Journal of Plant Science* **94**(3) 545-553

Gale MD and Ainsworth CC (1984) The relationship between α-amylase species found in developing and germinating wheat grain. *Biochemical Genetics* **22** 1031-1036.

Gao F, Jordan MC and Ayele BT (2012). Transcriptional programs regulating seed dormancy and its release by after-ripening in common wheat (Triticumaestivum L.). *Plant Biotechnology Journal* **10** 465-476. doi:10.1111/j.1467-7652.2012.00682.x

Gao X, Hu CH, Li HZ and Yao YZ *et al.*, (2013). Factors affecting pre-harvest sprouting resistance in wheat (Triticumaestivum L.). *Journal of Animal and Plant Science* **23**(2) 556-565.

Gerjets T, Scholefield D, Foulkes MJ and Lenton JR *et al.*, (2010) An analysis of dormancy, ABA responsiveness, after-ripening and pre-harvest sprouting in hexaploid wheat (Triticumaestivum L.) caryopses. *Journal of Experimental Botany* **61**(2) 597-607.

Ghanbari M and Mir B (2013) Genetic analysis of pre-harvest sprouting resistance in wheat cultivar (Triticumaestivum L.). *International Journal of Agronomy and Plant Production* **9** 2260-2266.

Groos C, Gay G, Perretant MR and Gervais L *et al.*, (2002). Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in white × red grain bread wheat cross. *Theoretical and Applied Genetics* **104** 39-47.

Hatcher DW and Symons SJ (2000). Influence of sprout damage on oriental noodle appearance as assessed by image analysis. *Cereal Chemistry* **77** 380-387.

Himi E, Yamashita Y, Haruyama N, Yanagisawa T, Maekawa M and Taketa S (2012). Ant28 gene for proanthocyanidin synthesis encoding the R2R3 MYB domain protein (Hvmyb10) highly affects grain dormancy in barley. *Euphytica* **188** 141-151.

Hurkman WJ, McCue KF, Altenbach SB, Korn A and Tanaka CK *et al.*, (2003). Expression of genes for starch biosynthesis is regulated by high temperature in developing wheat endosperm. *Plant Science* **164** 873-881.

Jacobcen JV, Barrero JM, Hughes T and Julkowska M *et al.*, (2013). Roles for blue light,jasmonate and nitric oxide in the regulation of dormancy and germination in wheat grain (*Triticumaestivum* L.). *Planta* **238** 121-138.

Jaiswal V, Mir RR, Mohan A, Balyan HS and Gupta PK (2012). Association mapping for pre-harvest sprouting tolerance in common wheat (Triticumaestivum L.). *Euphytica* **188** 89-102.

Jenner CF (1991). Effects of exposure of wheat ears to high temperature on dry matter accumulation and carbohydrate metabolism in the grain of two cultivars, Immediate responses. *Australian Journal of Plant Physiology* **18** 165-177.

Kaplan F and Guy CL (2004). ß-amylase induction and the protective role of maltose during temperature shock. *Plant Physiology* **135** 1674-1668.

Keeling PL, Bacon PJ and Holt DC (1993). Elevated temperature reduces starch deposition in wheat endosperm by reducing the activity of soluble starch synthase. *Planta* **191** 342–348.

Knox RE, Clarke FR, Clarke JM, Fox SL, DePauw RM and Singh AK (2012). Enhancing the identification of genetic loci and transgressive segregants for preharvest sprouting resistance in a durum wheat population. *Euphytica* **186** 193-206.

Kondhare KR, Hedden P and Kettlewell PS *et al.*, (2014). Use of the hormone-biosynthesis inhibitors fluridone and paclobutrazol to determine the effects of altered abscisic acid and gibberellin levels on pre-maturity α-amylase formation in wheat grains. *Journal of Cereal Science* **60** 210- 216.

Kulwal P, Ishikawa G, Benscher D, Feng Z, Xi Yu L and Jadhav A *et al.*, (2012). Association mapping for pre-harvest sprouting resistance in white winter wheat. *Theoretical and Applied Genetics* **125** 793–805.

Lin R, Horsley RD and Schwarz PB (2008) Associations between caryopsis dormancy, alpha-amylase activity, and pre-harvest sprouting in barley. *Journal of Cereal Science* **48** 446-456. doi:10.1016/j.jcs.2007.10.009

Lin RS, Horsley RD and Schwarz PB (2008). Associations between caryopsis dormancy, α- amylase activity, and pre-harvest sprouting in barley. *Journal of Cereal Science* **48** 446-456.

Lindblom H, Jonsson JO, Larssonraznikiewicz M and Salomonsson L (1989). Starch-degrading enzymes in some triticale, wheat and rye cultivars during kernel development. *Swedish Journal of Agricultural Research* **19** 65-72.

Liu S, Cai S, Graybosch R, Chen C and Guihua B (2008). Quantitative trait loci for resistance to pre-harvest sproutingin US hard white winter wheat Rio Blanco. *Theoretical and Applied Genetics* **117** 691-699.

Lunn GD, Major BJ, Kettlewell PS and Scott RK (2001). Mechanisms leading to excess alpha-amylase activity in wheat (*Triticum aestivum* L.) grain in the UK. *Journal of Cereal Science* **33** 313- 329.

Mares D and Mrva K (2007). Late-maturity *α*-amylase: Low falling number in wheat in the absence of pre-harvest sprouting. *Journal of Cereal Science* **47** 16-17. doi:10.1016/j.jcs.2007.01.005

Mares D and Mrva K (2008). Late-maturity alpha-amylase: low falling number in wheat in the absence of preharvest sprouting. *Journal of Cereal Science* **47** 6-17.

Mares D, Rathjen J, Mrva K and Cheong J (2009). Genetic and environmental control of dormancy in white-grained wheat (*Triticum aestivum* L.). *Euphytica* **168** 311-318.

Masojc P and Milczarski P (2009). Relationship between QTLs for pre-harvest sprouting and alpha-amylase activity in rye grain. *Molecular Breeding* **23** 75-84.

Masojc P, Kosmala A and Perlikowski D (2013). Proteomic analysis of developing rye grain with contrasting resistance to pre-harvest sprouting. *Journal of Applied Genetics* **54** 11-19.DOI:10.1007/s13353-012-0127-8

McCaig TN, Gan YT, Clarke P, Clarke JM and DePauw RM (2006). Kernel colour changes associated with field weathering of spring wheat. *Canadian Journal of Plant Science* **86** 371-377.

Morris CF, Geng H, Beeher BS and Ma D (2013). A review of the occurrence of grain softness -1genes in wheat (*Triticum aestivum* L.). *Plant Molecular Biology* **83** 507-521.

Nakamura S, Abe F, Kawahigashi H and Nakazono K *et al.*, (2011). A Wheat Homolog of mother of FT and TFL1 Acts in the Regulation of Germination. *American Society of Plant Biologists Plant Cell* **23**(9) 3215-3229. DOI 10.1105/tpc. 111.088492.

Oszvald M, Tamas L, Shewry PR and Tosi P (2014). The trafficking pathway of a wheat storage protein in transgenic rice endosperm. *Annals of Botany* **113** 807-815.

Potokina E, Sreenivasulu N, Michalek LAW and Graner A (2002) Differential gene expression during seed germination in barley (*Hordeum vulgare* L.). *Functional & Integrative Genomics* **2** 28-39.

Rentzsch S, Podzimska D, Voegele A, Imbeck M, Mu¨ller K, Linkies A and Leubner-Metzger G (2012). Dose- and tissue-specific interaction of monoterpenes with the gibberellin-mediated release of potato tuber bud dormancy, sprout growth and induction of α- amylases and ß-amylases. *Planta* **235** 137-151. doi:10.1007/ s00425-011-1501-1

Shaik SS, Carciofi M, Martens HJ, Hebelstrup KH and lennow AB (2014). Starch bioengineer ring affects cereal grain germination and seedling establishment. *Journal of Experimental Botany* **65** 1- 14.

Shewry PR, Charmet G, Branlard G and Lafiandra D *et al.*, (2012). Developing new types of wheat with enhanced health benefits. *Trends in Food Science & Technology* **25**(2) 70-77.

Shewry PR, Piironen V, Lampi AM and Edelmann M *et al.*, (2010). The health grain wheat diversity screen: Effects of genotype and environment on phytochemicals and dietary fiber components. *Journal of Agricultural and Food Chemistry* **58** 9291-9298.

Shiferaw B, Smale M, Braun HJ and Duveiller E *et al.*, (2013). Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. *Food Security* **5** 291-317. DOI:10.1007/s12571-013-0263-y

Shockravi S, Almgren A, Carlsson NG and Sandberg AS (2012). Dephytinisation of Sangak and Barbari bread made from different extraction rate flours increases iron and zinc bioaccessibility in Caco-2 cells. *International Journal of Food Science & Technology* **47** 2252-2258.

Singh AK, Knox RE and Clarke JM *et al.*, (2014). Genetics of pre-harvest sprouting resistance in a cross of Canadian adapted durum wheat genotypes. *Molecular Breeding* **33** 919-929.

Singh AK, Knox RE, Clarke JM and Clarke FR *et al.*, (2014). Genetics of pre-harvest sprouting resistance in a cross of Canadian adapted durum wheat genotypes. *Molecular Breeding* **33** 919–929.

Singh R, Matus-Cadiz M, Baga M, Hucl P and Chibbar RN (2010). Identification of genomic regions associated with seed dormancy in white-grained wheat. *Euphytica* **174** 391-408.

Tashiro T and Wardlaw IF (1989). A comparison of the effect of high temperature on grain development in wheat and rice. *Annals of Botany* **64** 59-65.

Thitisaksakul M, Jimenez RC, Arias MC and Beckles DM (2012) Effects of environmental factors on cereal starch biosynthesis and composition. *Journal of Cereal Science* **56** 67- 80.

Wang XG, Ren JP and Yin J (2008). The mechanism on wheat pre-harvest resistant sprouting. *China Agriculture Science* **24** 243-250.

WHO (2003). Diet. Nutrition and the prevention of chronic diseases. In: *WHO Technical Report Science* (world health organization of the food and Agriculture organization of the United Nation, Geneva).

Wrigley C (2006). Late-maturity α-amylase apparent sprout damage without sprouting. *Cereal Foods World* **51** 124-125.

Wu Y, Hu HQ, Wang G, Zhang YZ and Ji J (2002). Relationship between α-amylase activity and resistance of pre-harvest sprouting in spring wheat. *Journal of Jilin Agricultural University* **7** 5-88.

Xing J, Symons S, Shahin M and Hatcher D (2010). Sprouting detection at early stages in individual CWAD and CWRS wheat kernels using SWIR spectroscopy. *Sensing and Instrumentation for Food Quality and Safety* **4** 95-100.

Yang Y, Zhang CL, Liu SX, Sun YQ, Meng JY and Xia LQ (2014) Characterization of the rich haplotypes of *Viviparous-1A* in Chinese wheats and development of a novel sequence-tagged site marker for pre-harvest sprouting resistance. *Molecular Breeding* **33** 75-88.

Zhang Y, Miao X, Xia X and He Z (2014). Cloning of seed dormancy genes (TaSdr) associated with tolerance to pre-harvest sprouting in common wheat and development of a functional marker. *Theoretical and Applied Genetics* **127** 855-866.