

Induction of alpha-Amylase in Wheat Grain Cultivars as an Indicator of Resistance to Pre-harvest Sprouting

Aidar A. Khakimzhanov, Vladimir A. Kuzovlev, Nurgul S. Mamytova, Dinara A. Shansharova, Oleg V. Fursov

Abstract—The influence of humidity and low temperature on the α -amylase activity and isoenzyme composition of grains of different wheat varieties have been studied. The identified samples of varieties have significant difference in the level of enzyme induction under the impact of high humidity and low temperature. It is proposed to use this methodological approach for testing genotypes and wheat breeding lines for resistance to pre-harvest sprouting (PHS).

Keywords— α -Amylase, isoenzymes, wheat, pre-harvest sprouting

I. INTRODUCTION

IN a number of climatic zones of Kazakhstan in the adverse weather conditions such as high air and soil humidity (in the rainy seasons) the PHS has been observed in the wheat grain during the ripening and harvesting periods, which results in a significant deterioration in baking properties of wheat, and even utter uselessness of wheat for bakery in some cases [1]. One of the most distinctive features of sprouted grain is the increased α -amylase activity [2]. Germination of grain during maturity or storage period is induced by a synthesis of specific forms of the enzyme, termed the "malt" or "germination" α -amylase, which leads to hydrolysis of starch granules, the formation of water soluble dextrans, and, ultimately, the crude baked bread [3], [4]. It is established that exposure of wheat to PHS depends on the wheat genotype [5]. This fact opens the prospects for breeding for resistance to this factor. Together with the breeders from Kazakh Research Institute of Agriculture, based on electrophoretic detection of "germination" α -amylase of more than 200 samples of wheat we have obtained a PHS-resistant cultivar, *Lutescens 70*.

Although, as a number of studies in recent years show, a resistance or a susceptibility to germinate in wheat grain is a multifactorial trait, which is associated not only with non-controlled synthesis of "germination" α -amylase in the maturing grain. A number of Australian scientists' studies [6], [7] demonstrated the existence of an enzyme with high isoelectric points (pI) in a number of late maturity α -amylase (LMA) genotypes.

These authors found that the LMA form of the enzyme in wheat is a result of a genetic defect that leads to the accumulation of high activity α -amylase with high pI in the ripening seeds in the absence of conditions for germination (high humidity) or other environmental factors. In the UK the LMA enzyme is also termed as pre-maturity α -amylase (PMAA) [8]. These authors emphasize that the LMA or PMAA enzyme is a result of a genetic defect that is present in some wheat genotypes, which can be spread around the world through negligent breeding programs. This α -amylase, synthesized in the maturing grain, maintains or increases its activity due to storage temperature (about +12⁰ C), which subsequently leads to low falling number (FN) index and adversely affects on the quality of the end-product i.e. bread [9], [10].

In addition to the revealed factors of the resistance/susceptibility to PHS we have identified another factor – a low content of phytohormone abscisic acid (ABA) in grain [11]. As it is known, the ABA being antagonist to gibberelic acid, inhibits the synthesis of "germination" α -amylase and contributes to the preservation of seed in the dormant state [12]. Our studies have shown that PHS-resistant *Lutescens 70* cultivar contains more ABA than susceptible *Novosibirskaya 67*.

Thus, this brief review points to a genetic dependence and multifactorial resistance to PHS. The significance of the research topic is confirmed by the development of international and national genetic programs aimed at understanding this phenomenon and the search for resistant genotypes [13], [14].

In this work we have investigated the influence of artificial model conditions that encourage the sprouting process in close to the climatic conditions of main grain producing regions of Kazakhstan on the activity and isoenzyme spectra of α -amylase of various soft wheat grains in order to identify varieties and breeding lines that are resistant to pre-harvest sprouting.

II. MATERIALS AND METHODS

A. Seed Material

The study examined several varieties of soft spring wheat: (*Triticum aestivum* L.), cultivated in Kazakhstan (Saratovskaya 29, Kazakhstanskaya 10, Kazakhstanskaya rannya, Kaiyr, Almaken, Yrym, *Lutescens 70*, *Lutescens 157*, *Lutescens 314*, *Lutescens 462*), and the promising breeding

A.A. Khakimzhanov, V.A. Kuzovlev, N.S. Mamytova and O.V. Fursov are with the Institute of Molecular Biology and Biochemistry, Almaty, Kazakhstan (e-mail: aidar1611@gmail.com).

D.A. Shansharova is with the Faculty of Food Technology Almaty Technological University, Kazakhstan.

lines (no.132 winter, no.132 spring, no.138 winter, no.132 spring) harvested in 2007 from the fund of Kazakh Research Institute of Agriculture.

B. Experimental Conditions

To modeling the provocative conditions of sprouting, freshly harvested wheat heads of fully ripe stage of the studied cultivars and breeding lines were placed in a climate chamber KBWF240 (Binder, Germany) with controlled temperature and humidity. Day and night time temperatures were 18⁰ and 10⁰C respectively at a constant humidity of 80%. The wheat heads served as controls, which were stored at room temperature. The procedures of treatment (humidification and cooling) imitated the weather conditions with low temperature and high humidity during the harvest season, typical for the northern regions of Kazakhstan.

C. Alpha-Amylase Studies

At the day 3, 7 and 11 the total amylase and α -amylase activity in the control and experimental (subjected to treatment) grains have been determined by starch-iodine method [15]. Isoelectric focusing (IEF) of α -amylase was carried out on 1 mm plates 5% polyacrylamide gel (PAG) in a gradient of Pharmalyte pH 4-9 (Pharmacia, Sweden). Upon the completion of IEF PAG-plates were incubated in 1.5% starch solution for 1 hour at +4⁰C, followed by staining of zones activity of the enzyme by J₂/KJ solution.

III. RESULTS AND DISCUSSIONS

The variability of the grain amylase activity after the treatment by low temperature and high humidity at the day 3, 7 and 11 has been investigated in the study. The data presented in TABLE 1 indicates the stimulating effect of grain treatment on the overall ($\alpha + \beta$) amylase activity. Moreover, a significant (more than 10-fold) increase in amylase activity in grain was observed only in some varieties (Saratovskaya 29, Kazakhstanskaya 10, Lutescens 462 and no.138 winter). Other variety samples (Yrym, Lutescens 70 and Lutescens 314) differed by significantly lesser increase (2.0 - 3.5 times) in total amylase activity by the day 11 of the treatment.

As noted, the effect of sprouting, caused by the conditions of ripening or storage of wheat grain is associated with the synthesis of α -amylase. In this regard, a study also examines the variability of this enzyme activity by the action of low temperature and high humidity (TABLE 2).

The data presented in TABLE 2 also shows the growth of α -amylase activity caused by the treatment. In control samples the activity of α -amylase was minimal and did not change significantly from the storage period.

The greatest increase in activity of α -amylase compared to control at the 11th day of treatment was showed by the following cultivars and breeding lines: Saratovskaya 29 (412 fold), Kazakhstanskaya 10 (148 fold), Lutescens 462 (173 fold), no.132 winter (116 fold), no.132 spring (105 fold) and Kaiyr (140 fold). More stable and resistant to treatment were the following cultivars: Yrym (40 fold), Lutescens 70 (93 fold), Lutescens 314 (21 fold), Lutescens 157 (74 fold) and no.

138 winter (32 fold). From the TABLE 2 it can be inferred that there are differences in the resistance of wheat genotypes to sprout, i.e., the presence of some endogenous factors that prevent the uncontrolled synthesis of α -amylase in provocative conditions. In order to determine the contribution of different groups of α -amylase isoenzymes in forming the overall amylase and α -amylase activity under provocative conditions we studied the enzyme IEF spectra by the stages of treatment (Fig.1, a). In the spectra of control samples only the activity of low pI isoenzymes (Group A), the so-called "green" forms of α -amylase [16] has been revealed. The presence of this group in a resting grain of control samples is due to the fact that the grain was taken for analysis in the late stages of ripeness, so these isoforms probably did not went into a latent state. Increased activity of α -amylase after exposure to provocative conditions associated with the induction of "germination" α -amylase isozymes of groups B and C (Fig.1, b, c, d). Moreover, it is clear that the samples differed in the dynamics of these α -amylase isoenzymes synthesis.

The greatest differences between the studied cultivars and breeding lines in the isozyme spectra of α -amylase induced by treatment, were observed on the day 7 (Fig.1, c). The maximum activity of "germination" α -amylase isoenzymes were exhibited by Saratovskaya 29, Kazakhstanskaya 10, Lutescens 462, which differed from other varieties by the high enzyme activity (TABLE II). Less heterogeneous were isozyme spectra of Yrym, Lutescens 70, Lutescens 314, Lutescens 157 and no. 138 winter. By the end of the treatment period (the day 11) the differences between the samples for isozyme composition of "germination" α -amylase have been leveled due to high specific activity of the enzyme at this stage of treatment. Despite this, the differences in the isozyme compositions of α -amylase with high pI (Group C), which exhibited its activity at the day 7 of treatment, were still visible (Fig.1, c, d). It is not excluded that it may be associated with the presence of above mentioned LMA form of the enzyme in certain genotypes. More detailed studies on the possibility of the presence of LMA form of α -amylase in genotypes of wheat grown in Kazakhstan are the subject of our further work.

IV. CONCLUSION

Thus, among the studied wheat cultivars we have identified resistant and non-resistant to the external factors of α -amylase induction, i.e. to the PHS or sprouting during the storage. Non-resistant to these effects varieties and breeding lines differed by accelerated dynamics of the synthesis of "germination" α -amylase isoenzymes. More resistant to the induction of the enzyme cultivars can provide a good basis for breeding for resistance to sprouting.

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TABLE I
CHANGES IN TOTAL AMYLASE ACTIVITY IN GRAINS OF DIFFERENT WHEAT CULTIVARS AND LINES UNDER THE INFLUENCE OF HIGH HUMIDITY AND LOW TEMPERATURE

Wheat cultivar	Amylolytic activity, U/ml h				
	Control Day 0	Day 3	Day 7	Day 11	Control Day 11
Saratovskaya 29	212.80 ± 78.84	820.20 ± 34.44	775.80 ± 24.03	3638.40 ± 167.35	227.20 ± 12.03
Kazakhstanskaya 10	241.60 ± 10.63	754.20 ± 25.64	706.80 ± 27.92	2534.40 ± 101.38	235.60 ± 9.4
Kazakhstanskaya rannya	235.60 ± 8.95	583.60 ± 24.51	784.00 ± 34.49	1407.60 ± 54.95	224.80 ± 7.34
Kaiyr	220.00 ± 8.80	567.60 ± 18.67	573.00 ± 23.49	1519.20 ± 54.98	229.40 ± 8.47
Almaken	220.40 ± 9.24	637.20 ± 22.93	581.00 ± 22.07	1256.40 ± 52.12	205.20 ± 7.79
Yrym	222.40 ± 8.67	616.20 ± 24.03	525.60 ± 15.22	468.00 ± 21.06	222.10 ± 9.10
Lutescens 70	230.00 ± 9.20	622.20 ± 26.75	536.80 ± 16.08	773.80 ± 30.56	220.40 ± 7.46
Lutescens 462	228.40 ± 8.91	729.60 ± 30.64	853.80 ± 24.02	3048.00 ± 975.36	226.00 ± 10.39
Lutescens 314.	217.40 ± 9.78	679.20 ± 24.45	629.40 ± 22.37	684.00 ± 33.65	225.80 ± 6.13
Lutescens 157	209.40 ± 8.38	414.00 ± 16.97	667.20 ± 24.01	1128.00 ± 43.99	208.60 ± 7.48
no. 132 winter	230.00 ± 9.68	1600.80 ± 62.43	1515.60 ± 57.51	2246.40 ± 89.84	229.60 ± 7.78
no. 132 spring	243.00 ± 10.45	1736.40 ± 69.44	1207.20 ± 48.28	2431.20 ± 92.37	236.60 ± 8.28
no. 138 winter	229.00 ± 8.24	1286.40 ± 50.15	1090.80 ± 45.76	2580.00 ± 105.78	226.60 ± 9.04
no. 138 spring	268.20 ± 9.92	899.60 ± 34.16	966.00 ± 41.73	2071.20 ± 86.98	246.60 ± 9.84

TABLE II
CHANGES IN TOTAL α -AMYLASE ACTIVITY IN GRAINS OF DIFFERENT WHEAT CULTIVARS AND LINES UNDER THE INFLUENCE OF HIGH HUMIDITY AND LOW TEMPERATURE

Wheat cultivar	Amylolytic activity, U/ml h				
	Control Day 0	Day 3	Day 7	Day 11	Control Day 11
Saratovskaya 29	4.72± 0,18	26.18 ± 1,07	88.02 ± 2,81	1713.60 ± 71,97	4.16± 0,18
Kazakhstanskaya 10	5.66± 0,22	15.14 ± 0,59	62.28 ± 19,02	888.00 ± 38,18	5.99± 0,25
Kazakhstanskaya rannya	4.89± 0,16	3.07 ± 0,13	4.62 ± 0,07	418.08 ± 16,30	3.91±0,15
Kaiyr	3.82± 0,14	7.40 ± 0,27	6.21± 0,23	525.12 ± 21,03	3.76± 0,13
Almaken	5.57± 0,13	5.12 ± 0,20	5.61± 0,22	321.52 ± 10,44	3.71± 0,13
Yrym	4.74± 0,19	3.19 ± 0,14	5.00 ± 0,19	126.72 ± 4,43	3.18± 0,13
Lutescens 70	4.30± 0,14	2.65 ± 0,11	2.00 ± 0,12	432.00 ± 16,76	4.56± 0,20
Lutescens 462	4.85± 0,18	12.28 ± 0,51	13.76 ± 0,53	752.40 ± 3,08	4.34± 0,18
Lutescens 314	3.78± 0,17	6.42 ± 0,24	6.81± 0,27	83.76 ± 3,22	4.08± 0,16
Lutescens 157	7.49± 0,30	5.64 ± 0,25	37.26 ± 1,56	23.80 ± 0,95	3.13± 0,13
no. 132 winter	8.01± 0,32	207.00 ± 8,52	203.76 ± 8,35	711.60 ± 29,86	6.14± 0,27
no. 132 spring	8.13± 0,29	269.60 ± 10,29	249.50± 9,48	629.40 ± 27,67	6.06± 0,24
no. 138 winter	8.25± 0,31	157.32 ± 5,65	121.50 ± 5,10	125.40 ± 52,66	3.93± 0,13
no. 138 spring	8.13± 0,31	169.20 ± 6,63	209.70 ± 8,80	645.00 ± 26,44	7.82± 0,30

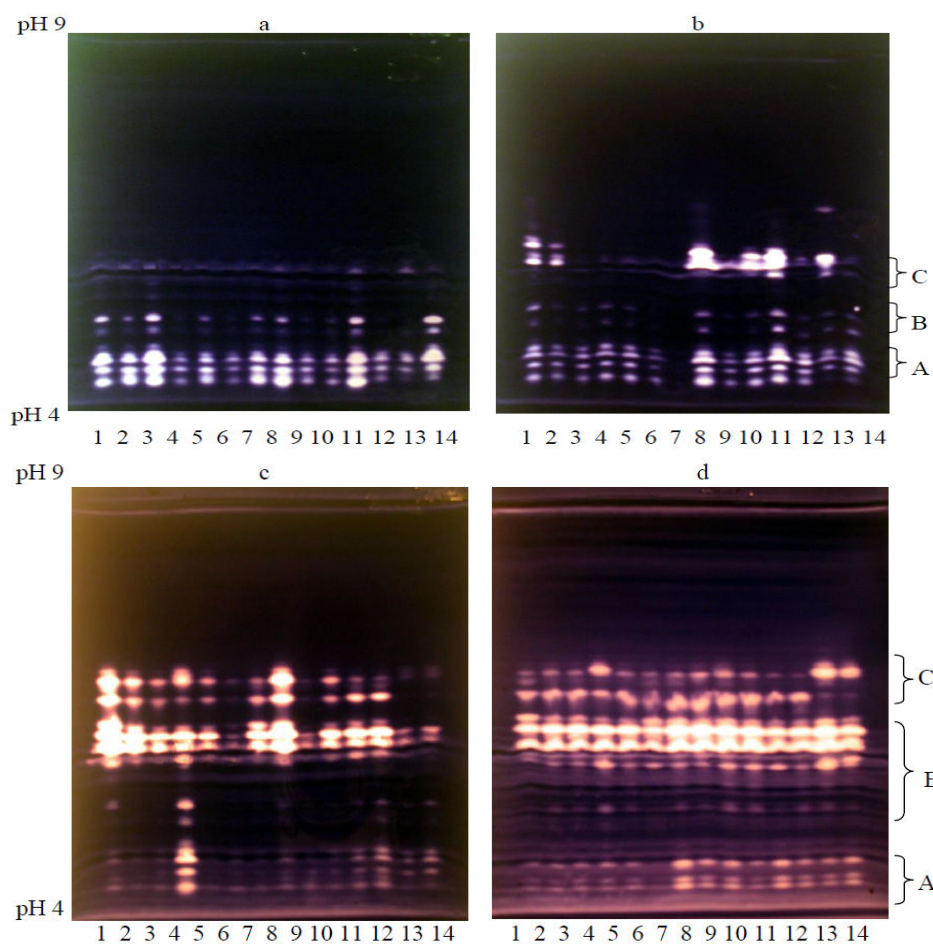


Fig. 1 Isoelectrofocusing of α -amylase of different wheat cultivars and lines
a – control (no treatment); b, c and d – day 3, 7 and 11 of seed treatment respectively by low temperature and high humidity;
1-14 – wheat cultivars: Saratovskaya 29, Kazakhstanskaya 10, Kazakhstanskaya rannya, Kaiyr, Almaken, Yrym, Lutescens 70, Lutescens 462, Lutescens 314, no. 132 winter, no. 132 spring, no. 138 winter, no. 138 spring.
A, B and C – isoenzymic groups of α -amylase