Conference Proceedings

The 3rd International Conference on Bioscience and Biotechnology

(BioTech 2018)

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Colombo, Sri Lanka

Committee of the BioTech 2018

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MESSAGE FROM THE HOSTING PARTNER BioTech 2018

Dear Participants,

The University of Kent is delighted to welcome you to the 3rd International Conference on Bioscience and Biotechnology 2018, Colombo, Sri Lanka, as a hosting partner of the event. This meeting offers an exciting opportunity for the University of Kent to help foster and develop new interactions between research scientists to share knowledge and instigate new collaborations. The development of biotechnology, such that precision genome editing can be undertaken and large scale sequencing is becoming a 'routine' tool of the trade, has given scientists an unparalleled ability to manipulate and harness the power of biosciences and apply this to health, agriculture and environmental issues (to name but a few) whilst ensuring such tools are used in a responsible manner. The meeting here brings together delegates from a wide range of disciplines to discuss their latest findings and interpretations across the bioscience and biotechnology remit, providing a forum for the exchange of knowledge and forging of new ideas.

We welcome you again to the conference and wish you a successful and productive meeting in Colombo.

MESSAGE FROM THE CONFERENCE CO-CHAIR BioTech 2018

Dear Participants,

It is with great pleasure that the I welcome you on behalf of the organizing committee to the the 3rd International Conference on Bioscience and Biotechnology 2018 held in the beautiful city of Colombo, Sri Lanka. This now established conference consists of 2 conference tracks, Application of Biochemistry and Molecular Biology to Biotechnology and General Biological Engineering. Within these two tracks there is a range of sessions and topics from biodrug discovery and manufacturing to bioremediation and from biological systems and models to nano-medicine. The full details of the wide and cross-disciplinary range of topics is detailed on the website and abstract handbook. The breath of topics covered reflects the fact that bioscience and biotechnology plays an important role in many facets of everyday life and offers the potential to solve local and global challenges that face us all.

The scientific sessions cover cutting edge new science in the areas outlined. The extensive program has been designed by the committee to give as many early career scientists the opportunity to presentation alongside the high profile and keynote speakers that we have attracted to the meeting. We thank all the speakers for agreeing to participate and those who submitted abstracts. The poster session also provides opportunities for authors to present their work and we encourage everyone to visit the posters and discuss the work with the authors.

The organizing committee would especially like to thank our sponsors. Their generosity helps support this conference. I would also like to thank the rest of the organizing committee for undertaking all their assigned tasks in a timely manner and working together so well.

Finally, the location, the scientific program, and the social events have been designed to encourage networking and enhance scientific discussion amongst participants. It is anticipated that the oral and poster presentations in this inspiring environment will generate new ideas and the initiation of fruitful novel research projects and collaborations. Participants and their engagement make a scientific meeting and it is up to us all to ensure this is a successful conference that we all reflect upon favorably. We thank you for participating in the conference and wish you a successful and productive meeting in Colombo.

Mark Smales
Conference Co-Chair BioTech 2018
Professor of Industrial Biotechnology,
Director of the Industrial Biotechnology,
University of Kent,
United Kingdom.

MESSAGE FROM THE CONFERENCE CO-CHAIR BioTech 2018

On behalf of the organizing committee, it is my honor and pleasure to welcome all research scientists, academics, postgraduate and graduate students from all over the world to Colombo, Sri Lanka for the 3rd International Conference on Bioscience and Biotechnology 2018. The conference will be held from 8th to 9th March under the theme **"**Pursuing innovation in Bioscience and Biotechnology" .

The word "biotechnology" has received its importance and significance in last two decades, which is just unprecedented. Probability and possibilities behind this kind of attention towards biotechnology is due to its unlimited potential to serve and benefit humanity. It has a significant impact in almost all the domains of human life, may it be health, environment, food and agriculture.

Bioscience and Biotechnology 2018 depends on generous knowledge donations of experts to promote the scientific discoveries. It will be committed to promote open, transparent, and international scientific exchange on the "New research and innovations in Bioscience and Biotechnology" and contribute to the improvement of efforts taken to tackle local and global impacts of Biological Science.

The conference is organized as a set of main tracks; Application of Biochemistry and Molecular Biology to Biotechnology, General Biological Engineering, Biomedical Engineering and Bioinformatics, Computational Biology for Biotechnology and other related tracks Biostatics, Biometric, Bio measurement, Biomechanics, Biophysics, and System Biology. It focuses on importance of Bioscience and Biotechnology through the keynotes speeches, Plenary speeches, invited talks, technical programs and workshops.

I hope this two days of scientific discussions and presentations by leading experts in the World as well as new research carried out by young scientist will provide a perfect amalgam for novel discovery in both basic and applied research in the Biotechnology. The outcome of the conference will pave ways to many new developments in the field of Biotechnology Worldwide. I am sure that you all will tremendously benefit from the deliberation of this conference in a warm and friendly environment. Your presence and deliberation will make this conference remarkably successful in all aspects of Biotechnology.

I congratulate all of you for your commitment and active participation and wish you all the success.

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STUDY OF SPRING WHEAT VARIETIES FOR IDENTIFICATION OF FORMS RESISTANT TO CADMIUM

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Abstract: The purpose of our study was the identification of spring wheat varieties resistant to cadmium, a priority pollutant in the East Kazakhstan region. The experiments were carried out in the condition of model environmental pollution on 14-day sprouts of various spring wheat varieties, that were grown on a nutrient mixture, containing 0.1 mM of $CaSO_4$ and Cd ions at a concentration of 40 mg/l. Screening of spring wheat varieties under the conditions of the model experiment made it possible to identify resistant and sensitive genotypes. In terms of growth and accumulation of cadmium in the aboveground organs, the most resistant to cadmium spring wheat varieties are Samal and Kaiyr, the least resistant are the varieties Lutescens and Zhenis. In terms of growth and accumulation of cadmium in the roots, the most resistant varieties of spring wheat were Kazakhstan-15 and Kazakhstan Early, the most unstable – the variety Lutescens. Chlorophyll *a* is more sensitive to the action of cadmium ions than chlorophyll *в*. Cadmium-resistant varieties can be used in field studies of soil contamination with cadmium to identify promising forms that combine metal resistance with high yield and resistance to weather conditions.

Keywords: cadmium, spring wheat, growth parameters, cadmium content, chlorophyll content, sensitive and resistant genotypes

Background. Objectives and goals

Pollution of the environment, in particular by chemicals, is one of the most powerful factors of destruction of the biosphere components. At present, a large number of pollutants enter the biosphere. Among them, a significant place is occupied by heavy metals. Heavy metals are the most toxic among chemical elements [1].

One of the ways to obtain clean commercial products on soils contaminated with heavy metals is to create, and use in the production, technogenic resistant varieties of agricultural crops. The development of this method raises the initial task of studying gene pool of the cultivated and wild plants and allocation of donors, accumulating minimum amount of contaminants in the commercial part of the crop [2]. The evaluation of selection material and the direction of researches on the principles of the use of attributes, that allow to accumulate a minimum number of ecotoxicants, will make it possible to reduce terms of selection work [3,4]. In connection with the task of studying the gene pool of cultivated plants in conditions of technogenic pollution, the subject of the study was the screening of varieties of spring wheat for metal resistance in order to identify the promising types for cultivation in the East Kazakhstan region, and selection donors that accumulate minimum amount of pollutants.

Methods

The objects of research are different genotypes of spring wheat from the collection of the Kazakh Research Institute of Agriculture (KIA): Kazakhstan Early, Erythrospermum, Kaiyr, Lutescens, Zhenis, Kazakhstan-25, Samal, Samgau, Almaken, Kazakhstan-15.

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Plant cultivation. The experiments were performed on 14-day sprouts of various wheat varieties grown on a nutrient mixture containing 0.1 mM $CaSO_4$ and cadmium ions at a concentration of 40 mg/l (as a $CdSO_4$ salt). Samples were grown in the aquatic environment at t-20^0C during the day and 16^0C at night, with a 10-hour photoperiod, light intensity - 5 thousand lux, humidity - 60%. Growth parameters, tolerance index, cadmium content in roots and aerial organs and the content of chlorophyll *a* and *b* in sprouts were determined.

Determination of growth parameters and the tolerance index. Measurement of biometric indicators was carried out by generally accepted methods. The tolerance index or the Wilkins coefficient was calculated by the formula: $I_t=I_{me}/I_c$, where I_{me} is the root increment in the solution with studied metal, and I_c is the root increment on the solution without metal [5].

Determination of heavy metals. Cadmium in soil and plant samples were determined by atomic absorption method on the device A Analyst 300 of "Perkin Elmer" firm. Sample preparation was carried out using a heating unit «Hot Block» with the addition of concentrated nitric acid and hydrochloric acid at a temperature of 90 ± 5 ° C, in accordance with standard operating procedures. Weighed portion of sample was placed in disposable sample cups; 5 ml of 50% nitric acid and 0.5 ml of concentrated hydrochloric acid were added. Samples were mixed well to liquid clay condition, covered with a watch glass and placed in a heating block. The sample was heated to a temperature of 90 ± 5 ° C, and evaporated for 10-15 minutes without boiling. Then the sample was cooled, 5 ml concentrated HNO_3 was added and heated again for 30 minutes. Content of capacitances were evaporated without boiling at a temperature of 90 ± 5 ° C for approximately up to 5ml during 2 hours, avoiding foaming. After that the sample was cooled and the volume was adjusted to 50 ml with deionized water. To calibrate the device the calibration blank have been used, consisting of deionized water and 1% HNO_3 solution and standard samples of the company «High Purity". After calibration of the devise, readings of analyzed samples were taken. Accuracy of analysis performance was checked by the screening standard of the company «Merck».

The content of heavy metal in the sample was calculated according to the formula:

$$C\ mg / kg = \frac{C\ device \times V\ samp. \times FD}{M},$$

wherein

C device - devise reading (mg / l);

V samp. - final volume of samples (ml);

FD - dilution factor;

M - weighed sample (g).

Determination of chlorophyll content. The content of photosynthetic pigments was determined by direct spectrophotometry method of undiluted acetone extracts according to Holm-Wettstein [6,7,8]. Absorption spectras of the extracts were recorded on a spectrophotometer SPEKS SCP-705.

Results

Study of the effect of cadmium on the growth of spring wheat plants. A study of the genotypic specificity of wheat for resistance to heavy metals was carried out for cadmium, a priority pollutant in the East Kazakhstan region. Cadmium is one of the most toxic environmental pollutants for plants. Increased concentrations of cadmium in the root-inhabited environment cause plants to slow growth and development [9]. Our studies of the influence of cadmium on growth parameters of wheat seedlings in laboratory conditions showed that cadmium ions suppress plant growth (Figure 1).

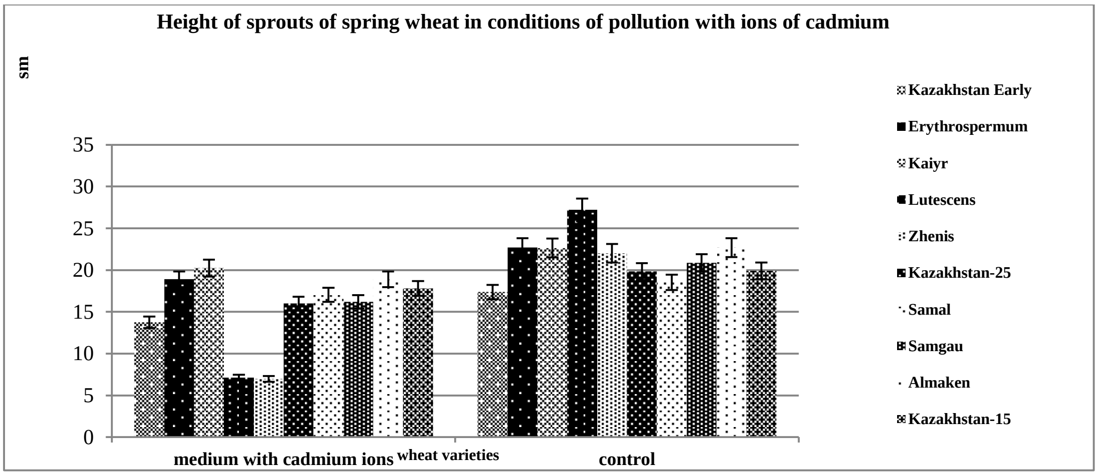

Figure 1. Influence of the presence of cadmium ions in the growth medium on the growth of seedlings of various varieties of spring wheat.

Our study of growth parameters of different wheat varieties have allowed to reveal specific features of cadmium toxicity depending on genotypic differences of wheat plants. In the study of genotypes from the KIA collection, it was shown (Figure 2) that according to the growth of the above-ground organs with a high cadmium concentration in the growing medium, the genotypes can be arranged as follows: Samal > Kaiyr > Kazakhstan-15 > Erythrospermum > Almaken > Kazakhstan-25 > Kazakhstan Early > Samgau> Zhenis> Lutescens (Figure 2). The most resistant to the adverse effect of cadmium ions, according to the growth indices of above-ground organs, were the varieties of spring wheat Samal, Kaiyr and Kazakhstan-15. In these genotypes, the growth inhibition of seedlings is 8.0, 10.6 and 10.7 percent, respectively, compared to the control.

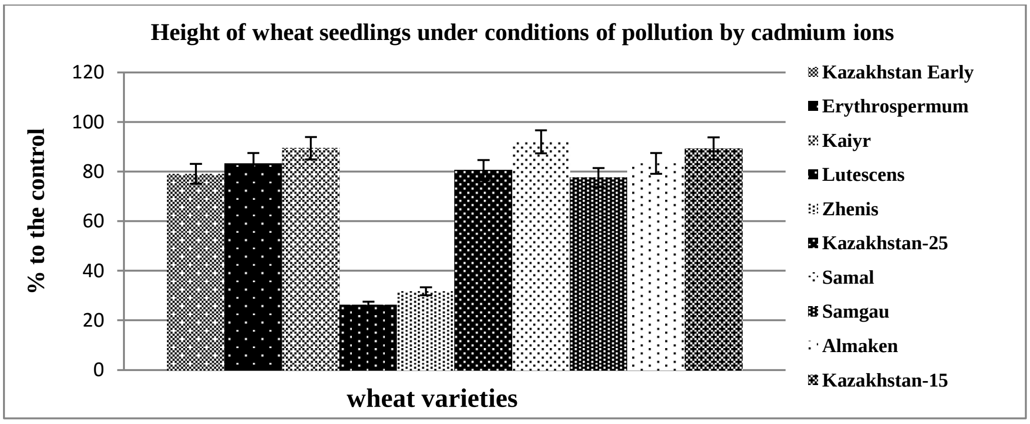

Figure 2. Reduction in the length of sprouts of different varieties of wheat (in percentage to the control) in conditions of environmental contamination with cadmium ions.

The average level of resistance to adverse effects of cadmium ions in spring wheat varieties Erythrospermum, Almaken, Kazakhstan-25, Kazakhstan Early, Samgau. By the growth of seedlings, the most unstable to the adverse effects of cadmium ions were spring wheat varieties Zhenis and Lutescens. In these varieties of spring wheat growth suppression seedlings compared to control occurs at 68.3 and 73.8 percent, respectively (figure 3).

Our studies of the effect of cadmium on the growth parameters of wheat roots under the conditions of the model experiment have shown that cadmium ions suppress the growth of plant roots. At the same time, the suppression of root growth is more significant than the suppression of growth of seedlings (figures 3 and 1).

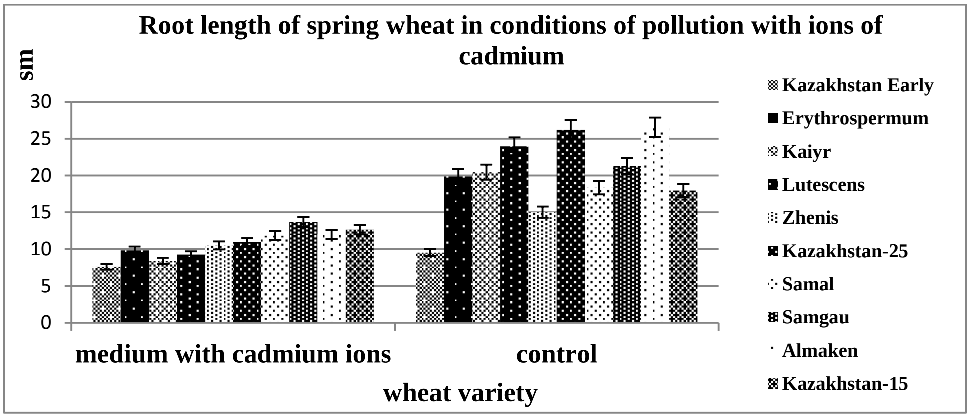

Figure 3. The influence of the presence of cadmium ions in the growing medium on the growth of roots of different wheat varieties.

Upon the root growth, when the salts of cadmium applied to the nutritional environment, genotypes can be arranged as follows: Kazakhstan Early > Kazakhstan-15 > Zhenis > Samal > Samgau > Erythrospermum > Almaken > Kazakhstan-25 > Kaiyr > Lutescens (figure 4).

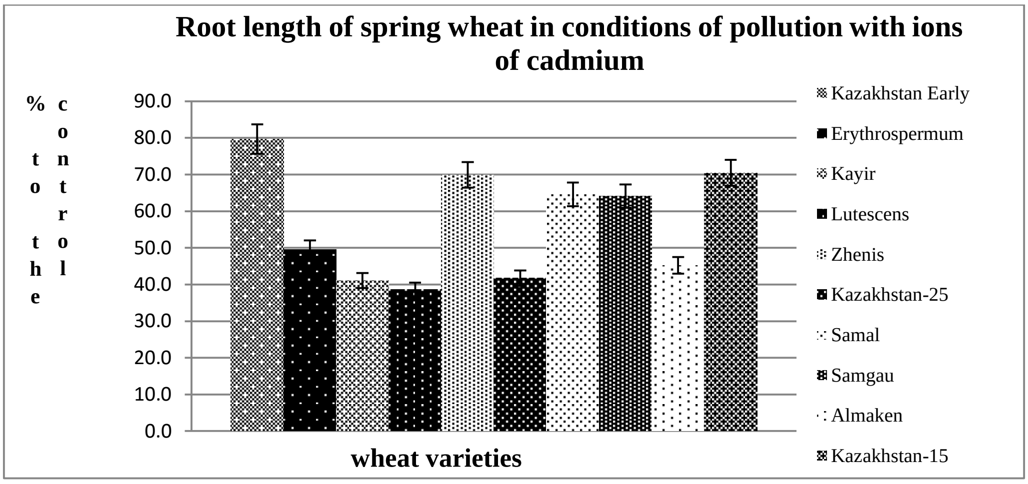

Figure 4. Reducing the length of roots of different wheat varieties (as a percentage of control) in terms of environmental pollution with cadmium ions.

The most resistant to the adverse action of ions of cadmium in the root system were varieties of spring wheat Kazakhstan Early, Kazakhstan-15 and Zhenis. Suppression of root growth compared with the control occurs by 20.3, and 29.5 and 30.1 percent. The average level of resistance of roots to the adverse effects of cadmium were detected in wheat varieties Samal, Samgau, Erythrospermum and Almaken. Less resistance to the adverse effect of cadmium showed varieties of spring wheat Kazakhstan-25 and Kaiyr. Root growth suppression compared to control occurs at 58.2 and 58.9 percent (figure 4).

The most unstable to the adverse effects of cadmium ions were the roots of plants of the variety Lutescens.. Suppression of root growth compared with the control occurs by 61.4 percent (see figure 4).

Wilkins coefficient was also determined, which shows the tolerance of plants to heavy metals (table 1).

Table 1. Wilkins coefficient or index of tolerance of wheat germ roots in conditions of contamination of the growing medium with cadmium ions.

Genotypes of wheat	**Kazakhstan Early**	**Erythrospermum**	**Kaiyr**	**Lutescens**	**Zhenis**	**Kazakhstan-25**	**Samal**	**Samgau**	**Almaken**	**Kazakhstan-15**
Im	7,60	9,85	8,40	9,25	10,50	10,95	11,84	13,65	12,00	12,65
Ic	9,54	19,87	20,44	23,95	15,02	26,22	18,34	21,29	26,52	17,95
It	0,80	0,50	0,41	0,39	0,70	0,42	0,65	0,64	0,45	0,70

Wilkins coefficient or the index of tolerance, with a high concentration of ions of cadmium in the growing medium, is the greatest in varieties of spring wheat Kazakhstan Early, Kazakhstan-15 and Zhenis, the average in varieties - Samal, Samgau, Erythrospermum and Almaken, smaller - varieties Kazakhstan-25 and Kaiyr, the least – in the variety Lutescens (table 3). According to the results of the study of root growth, when contaminated with cadmium ions, and according to the tolerance index, spring wheat varieties Kazakhstan early, Kazakhstan-15 and Zhenis can be identified as genotypes with the most stable root system to the adverse effects of this element. The most unstable to the adverse effects of cadmium ions, by the results of the determination of both parameters, were roots of plants of the Lutescens variety.

Thus, the varieties of spring wheat Samal, Kaiyr and Kazakhstan-15 were the most resistant to the action of cadmium ions by the growth of the aboveground organs, Zhenis and Lutescens varieties were the most unstable to the adverse action of this metal. By root growth, the most resistant to the action of cadmium ions were varieties Kazakhstan Early, Kazakhstan-15 and Zhenis, the most unstable was the variety of spring wheat Lutescens.

Accumulation and distribution of cadmium in the organs of spring wheat depending on varietal differences of samples in in the conditions of model test

Not being an essential element for plant life, cadmium is actively absorbed and easily transported through the plant, accumulates in all organs, has a cumulative effect [10]. Cadmium also accumulates in plant seeds. Thus, the content of a microelement in wheat grain is higher than in stems and leaves of plants [11, 12]. According to some researchers, the level of heavy metal cadmium in food is a problem of food security. Reducing cadmium in grain is one of the priorities of breeding programs [12].

Cadmium that enters surface organs may subsequently accumulate in the seeds, so we have focused on the translocation of cadmium in the aerial part of the plants. Varieties with the least content of cadmium in the aerial organs are subsequently used for field studies to identify promising for agricultural production forms, which are characterized by minimal cadmium accumulation in the seeds and in the same time characterized by high productivity and resistance to unfavorable climatic conditions of the environment.

The results of determination of cadmium content in the aerial organs of seedlings of the studied spring wheat varieties showed that at a dose of 40 mg/l of cadmium in the medium, the smallest amount in the aerial organs accumulate sprouts of spring wheat varieties Samal and Kayyr (figure 5).

The greatest number of cadmium in the aboveground organs, in these conditions of experiment, accumulate seedlings of spring wheat varieties Lutescens and Zhenis. The other genotypes occupy an intermediate position between them.

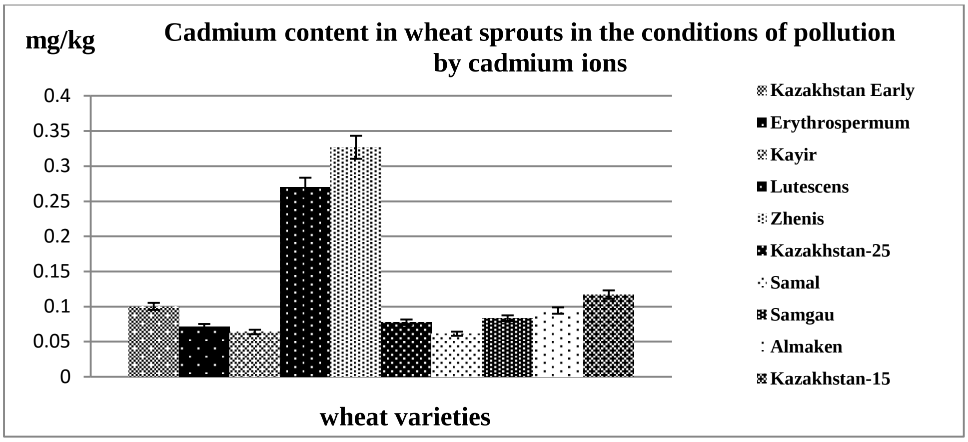

Figure 5. The content of cadmium in aboveground organs of different genotypes of wheat in terms of contamination of the growing environment with cadmium ions.

Investigation of cadmium content in roots of seedlings of various genotypes of spring wheat showed that at the dose of 40 mg/l in the environment, the smallest amount of it accumulated in the roots, the seedlings of spring wheat varieties Kazakhstan-15, Kazakhstan Early and Zhenis (figure 6).

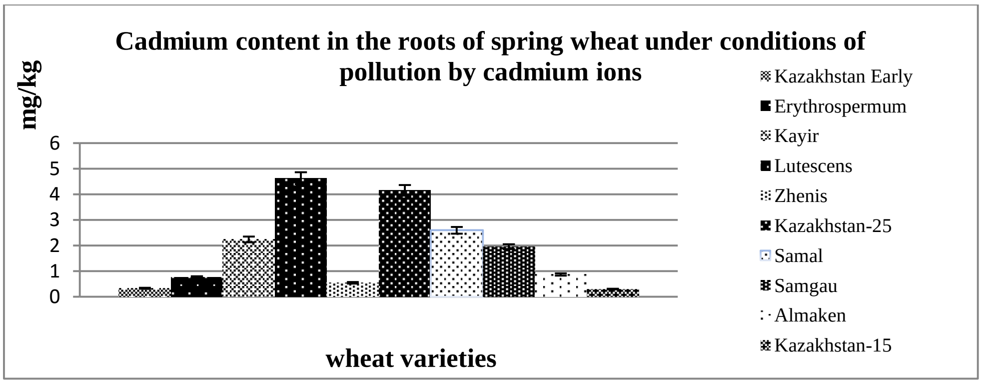

Figure 6. The content of cadmium in roots of various wheat genotypes in terms of contamination of the growing environment with cadmium ions.

Under these conditions, the greatest amount of cadmium accumulated in the roots of plants of the variety Lutescens.. The remaining varieties occupy an intermediate position between them.

According to the study of accumulation of cadmium in the aboveground organs of plants of different spring wheat varieties, varieties Samal and Kair can be selected as the genotypes resistant to translocation of cadmium in the aboveground organs. Accirding to the results of research of cadmium accumulation in aboveground organs of spring wheat it is possible to allocate varieties Kazakhstan-15, Kazakhstan Early and Zhenis as genotypes with the greatest root resistance to action of cadmium. Research of accumulation of cadmium in roots and aboveground organs, of accumulation of dry matter, and also of growth parameters of wheat seedlings of various genotypes allowed to reveal the most sensitive and stable genotypes. The most resistant to cadmium were varieties Samal and Kaiyr, and the most sensitive – spring wheat variety Lutescens.

Effect of cadmium on chlorophyll content in leaves of seedlings of different wheat varieties

Increased concentrations of cadmium in the root medium cause slowing of growth and development of plants [9], as well as disturbances in the course of basic physiological processes [13]. To understand the causes of negative impact of cadmium, it is important to know what specific anatomical, morphological, physiological and biochemical changes it causes in different parts of the plant, and, above all, in the leave, as the main organ of photosynthesis.

The study of the effect of cadmium on the photosynthetic apparatus of wheat plants showed the presence of certain changes in the number of basic forms of pigments. A slight increase in chlorophyll *a* was found in the presence of cadmium in most varieties (figure 7). The same data were obtained from other authors [14].

Earlier it was repeatedly noted that the photosynthetic apparatus of plants is one of the first to react to stress [15] and the state of the pigment system determines the efficiency of photoassimilation of CO_2 [16].

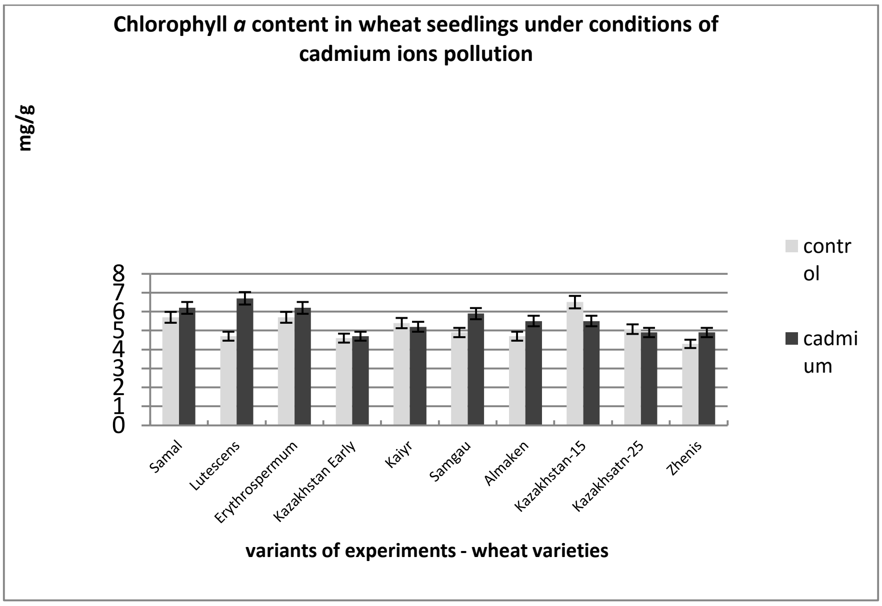

Figure 7. Effect of cadmium on chlorophyll a content in wheat leaves.

Our studies have shown that the content of chlorophyll *b* in seedlings of different varieties of wheat is different. In a medium with the addition of cadmium ions, the chlorophyll *b* content in the seedlings of different varieties of wheat is also uneven (figure 8).

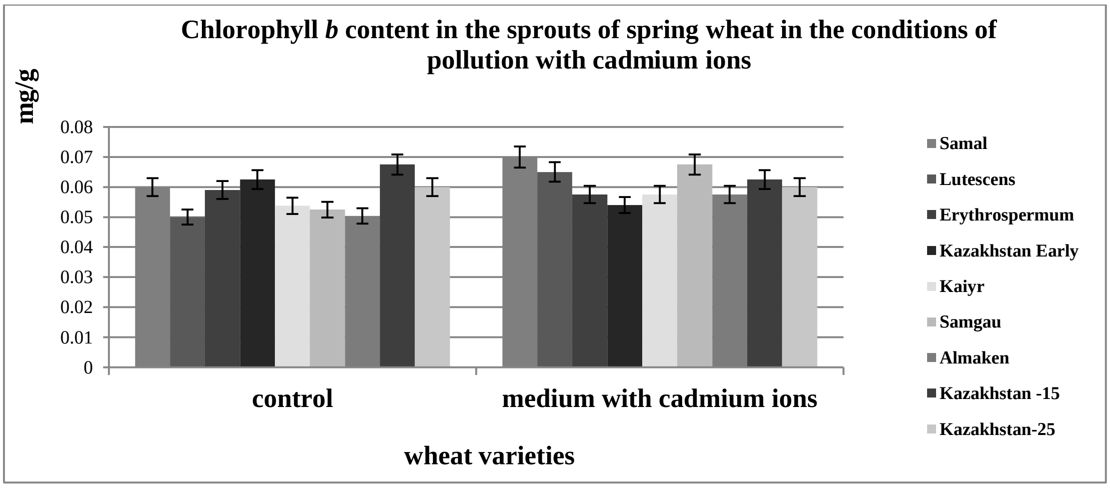

Figure 8. Chlorophyll b content in seedlings of different varieties of wheat in the medium contaminated with cadmium ions.

Erofeev E. A. and Naumova M. M. showed that cadmium sulfate in small concentrations leads to a decrease in the amount of chlorophyll a and b compared to the plant level of the control group. However, in the area of high salt concentrations, the amount of chlorophyll in T. Aestivum shoots increased to the level of the control group plants [17]. Also, for example, an increase in the total concentration of photosynthetic pigments is revealed in the leaves of clover, as a response to the presence of contaminants. The authors have made an assumption that the high total concentration of pigments of photosynthesis in the studied plants may be associated with the activation of photosynthetic apparatus, provoked by high energy costs, which are associated with the neutralization of the influence of pollutants [18].

At the same time, chlorophyll *a* content varied more strongly than chlorophyll b.

The ratio of chlorophyll *a/b* and the ratio of chlorophyll (*a* + *b*) to carotenoids can be significant indicators of the stability of the pigment complex to heavy metals [15].

These values are normally stable, but can change quickly under the influence of stress factors. Changes in the ratio of the content of photosynthetic pigments affect the activity of the photosynthetic apparatus, affecting the rate of accumulation of assimilates, growth and productivity of plants [15, 19].

Our data suggest that chlorophyll *a* is more sensitive to cadmium ions than chlorophyll *b* (figure 9). Similar data were obtained by other authors. Maleva M. G. with co-authors revealed that chlorophyll *a* is more sensitive to Cu and the co-action of Cu and Mn than chlorophyll *b* [20].

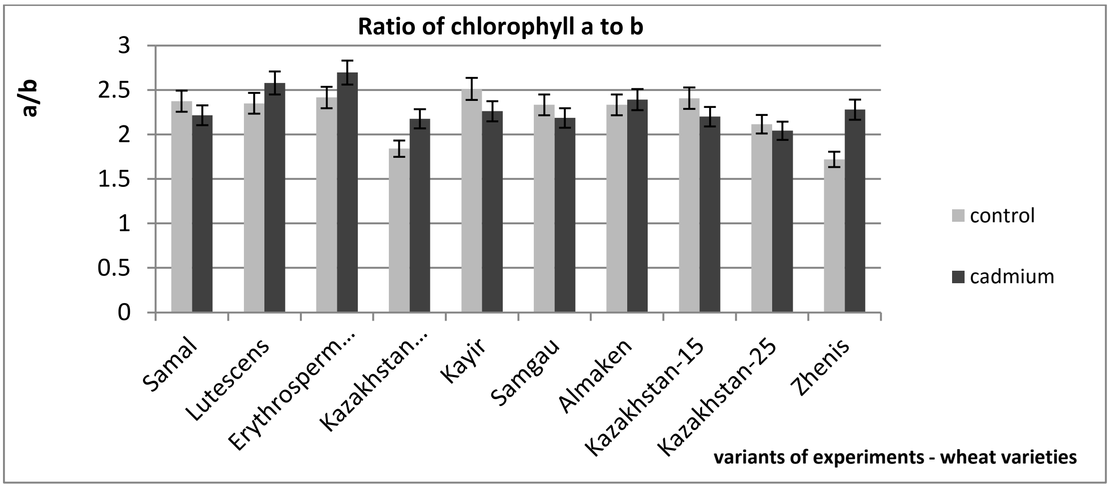

Figure 9. Changing the ratio of chlorophyll a / b in wheat leaves under conditions of contamination with cadmium ions.

The study of the action of cadmium on the content of chlorophyll in wheat varieties, contrasting on resistance to the studied heavy metal, revealed less variability of this indicator in more resistant genotypes to traclocale of cadmium in the aboveground organs compared to the more sensitive. Thus, the study of the impact of pollution with cadmium ions revealed that the number of chlorophyll *a* and *b* increases in the leaves of most varieties, which is probably due to the activation of the photosynthetic apparatus caused by high energy costs, which are associated with the neutralization of the influence of pollutants.

Conclusion

Based on the results, the following conclusions were made:

1. The most resistant to cadmium varieties were Samal and Kaiyr, and the most sensitive – spring wheat variety Lutescens.

2. The number of chlorophylls *a* and *b* increases in the leaves of most varieties, which, apparently, is due to the activation of the photosynthetic apparatus, provoked by high energy costs, which are associated with the neutralization of the influence of pollutants.

3. More resistant varieties showed less variability in the amount of chlorophyll compared with more sensitive.

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PROVING THE EFFICACY OF MANGIFERIN AS A NEUROPROTECTIVE DRUG USING DOCKING STUDIES

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Abstract: Mangiferin, a bioactive Xanthonoid known for its anti-oxidant & therapeutic activity, has been involved in a variety of potential pharmacological activities. The aim of this work is to prove the efficacy of mangiferin as a neuroprotective drug by docking it with β-amyloid precursor protein, which causes Alzheimer's. Alzheimer's disease (AD) is a progressive neurodegenerative disease, which is characterized by progressive cognitive deterioration and behavioral changes along with the chronic inflammation of neurons. Studies have shown that the deposition of amyloid-β (Aβ) peptide has resulted in the inflammatory changes such as astrocytosis and microgliosis. Based on the quantitative analysis of the molecular docking technique it was observed that mangiferin successfully docked with amyloid-β (Aβ) peptide. The ligand binding affinity was high due to the presence of many flexible docked conformers. The most stable conformer was having a final docked value of -12.421 with binding energy amounting to -4.11 kcal/mol. The *in silico* docking analysis demonstrates that mangiferin exhibits binding interactions with β-amyloid precursor protein that are potentially responsible for the inhibition of the protease enzyme which would be helpful in developing a neuroprotective drug for Alzheimer's Disease.

Keywords: Mangiferin, β-amyloid peptide (Aβ), Alzheimer's disease, AutoDock, Binding Energy

Introduction

Mangiferin (1,3,6,7-tetrahydroxyxanthone-C-2-*β*-D-glucoside, MGF), a constituent of *Curcuma amada* (Padmapriya *et al*. 2012) has been reported to have multiple pharmacological potentials like antioxidant activity, immunomodulatory effect, anti-inflammatory effect, effect of improvising dyslipidemia, therapeutic effect on periodontal disease and antidiabetic effect on both type 1 as well as type 2 diabetes (Guha *et al*.1996, Dar *et al.* 2005, Bhowmik *et al*. 2009, Niu *et al.* 2012, Duang *et al*. 2011). The importance of mangiferin lies with its radical scavenging activity (Miliauskas *et al*. 2004), inhibition of oxidative stress (Murunganandan *et al.* 2005) and its ability to form a complex with Iron (III)(Ghosal *et al*. 1996).

Alzheimer's disease (AD) is a progressive neurodegenerative disease, which is characterized by progressive cognitive deterioration and behavioural changes along with the chronic inflammation of neurons. AD is one form of dementia that gradually increases with time. Dementia is a loss of brain function that occurs with aging & certain diseases. AD is characterized by accumulation of the amyloid-β (Aβ) peptide and microtubule associated protein tan within the brain. Most often, AD is diagnosed in people over 65 years of age (Brookmeyer *et al* 1998). There are two types of onset- late and early. Genetic, biochemical and behavioural research suggest that physiological generation of a neurotoxic Aβ peptide from sequential amyloid precursor protein (APP) by proteolysis is the crucial step in the development of AD.

APP is a single-pass trans membrane protein expressed at high levels in the brain and metabolized in a rapid and highly complex fashion by a series of sequential proteases, including the intramembranous ϒ-secretase complex, which also processes other key regulatory molecules. Alzheimer's amyloid beta-protein precursor contains a Kunitz protease inhibitor domain (APPI) potentially involved in proteolytic events leading to cerebral amyloid deposition. Genetic studies of APP processing will be crucial to the development of therapeutic targets to treat Alzheimer's (O'Brien RJ & Wong PC, 2011).

Protein-ligand docking was performed between the molecular models of amyloid precursor protein (1AAP) and mangiferin (MGF).

Methods

Softwares: AutoDockTools (ADT) and AutoDock 4.2 was downloaded from www.scrippps.edu. PyMol was downloaded from https://pymol.org.

Protein preparation for docking: The 3D structure of Amyloid beta precursor protein (PDB ID-1AAP) was downloaded from RCSB Protein Data Bank (PDB) (https://www.rcsb.org/pdb/explore.do?structureId=1AAP), before initiating the docking simulations (Hynes *et al*. 1990). The original bimolecular structure was reduced to a unimolecular receptor by using PyMol. 1AAP was modified by adding polar hydrogens and then kept rigid in the docking process whereas all the torsional bonds of ligands were set free by Ligand module in AutoDockTools. Gasteiger charges were computed followed by creation of grid maps using AutoGrid 4.2.

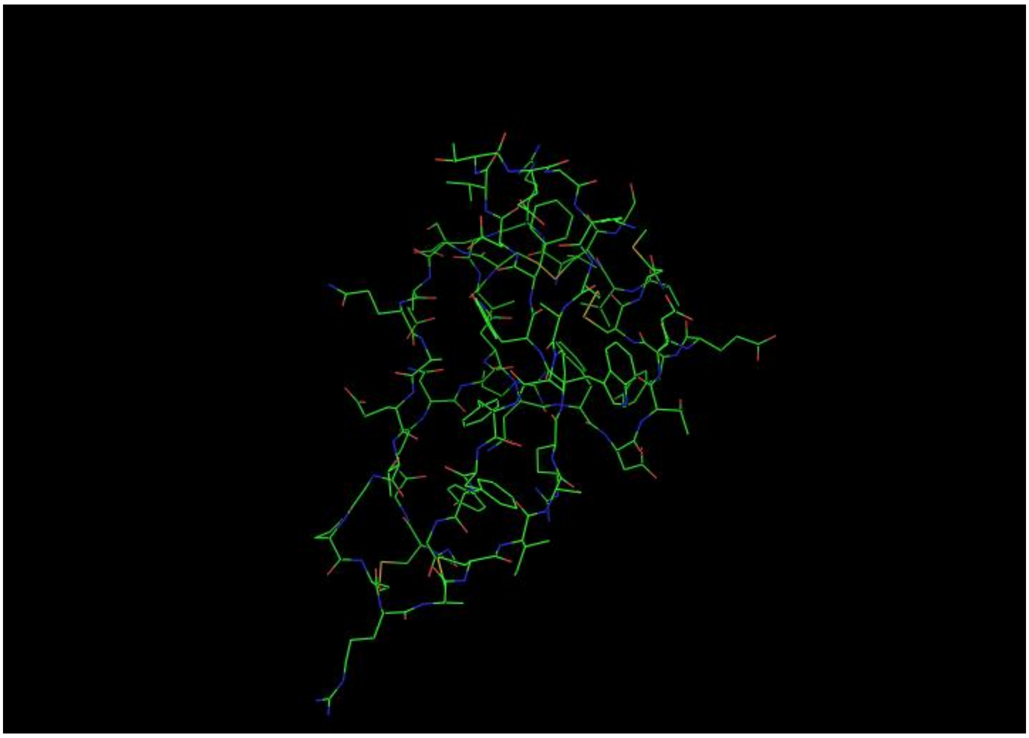


Figure 1: The three dimensional structure of amyloid-β precursor protein (PDB: 1AAP)

Ligand preparation for docking: The 3D structure of the ligand Mangiferin (CID-5281647) was downloaded from PubChem Open Chemistry Database (https://pubchem.ncbi.nlm.nih.gov/compound/Mangiferin) and was optimized using AutoDockTools.

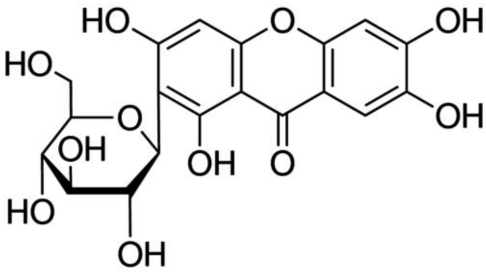


Figure 2: Parental Structure of Mangiferin

Molecular Docking: Molecular docking simulations were carried out using AutoDock 4.2 in AutoDockTools. The autodock 4.2 program was used to investigate the ligand binding with the amyloid beta precursor protein using a grid spacing of 0.5 Å and the grid points in X, Y and Z axis were set to 100 x 100 x 100 Å. The search was based on the Lamarkian Genetic Algorithm (Oprea et al., 2001) and the results were analysed using the binding energies. Top 10 conformers were ranked in order of increasing docking energies.

Results:

The docked positions of mangiferin into the binding site was explored using AutoDockTools software which is considered to be one of the most powerful tool for molecular recognition. The ligand mangiferin was used for the *in silico* docking analysis on amyloid-β precursor protein receptor (PDB: 1AAP). Molecular docking methods are commonly used for predicting binding modes to proteins and energies of ligands (Bikadi and Hazai, 2009). Docking was accomplished using AutoDock 4.2 which is a set of automated docking tools which was used to predict the binding affinity, activity and orientation of mangiferin to our docked target protein molecule. The analysis was based on the final score of docked conformations, free energy of binding values and Inhibition constant (Ki) of the conformers detected. Free energy of binding is calculated as a sum of four energy components (1) Final Intermolecular Energy *(Van der walls forces', hydrogen bonds, desolvation energy & electrostatic energy)*, (2) Total Internal Energy, (3) Torsional free energy and (4) Unbound system energy. Mangiferin was found to bind at various active sites of amyloid-β precursor protein with lowest estimated free binding energy found to be -4.11 kcal/mol along with the estimated inhibition constant (Ki) corresponding to 974.11μM (micromolar) at 298.15K temperature. The respective values for each conformer are listed in **Table 1** whereas the docked conformers are shown in **Figure 3 and 4**. The *in silico* experiment demonstrates that mangiferin binds with β-amyloid precursor protein thereby inhibiting the Kunitz protease domain which is potentially involved in the proteolytic events leading to cerebral amyloid deposition. Thus we can hypothesize that mangiferin may play an important role in inhibiting the protease activity present in the amyloid-β protein. Mangiferin can therefore be a promising candidate for the development and design of a neuroprotective drug with therapeutic activities.

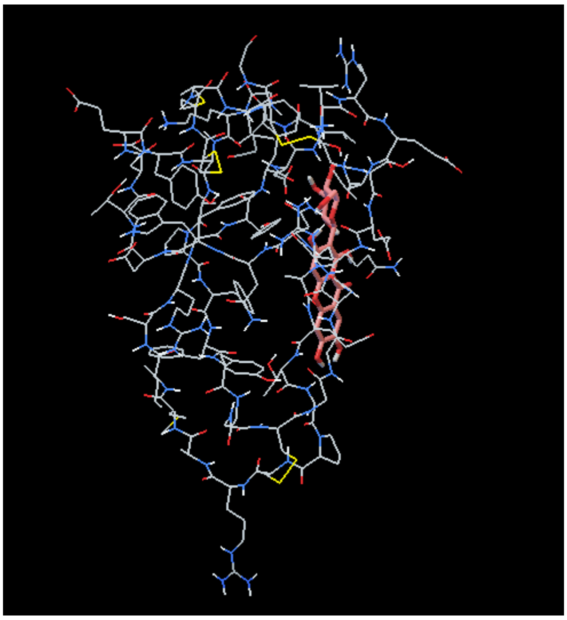
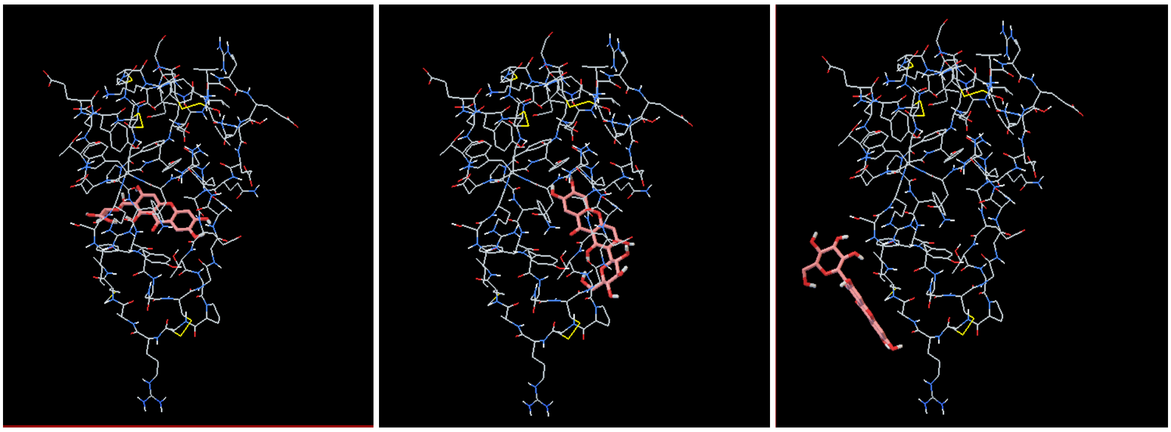
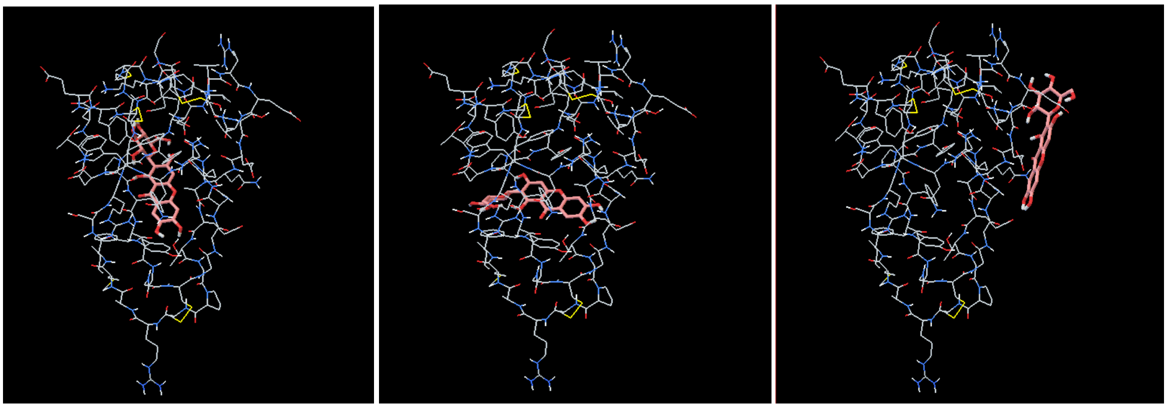


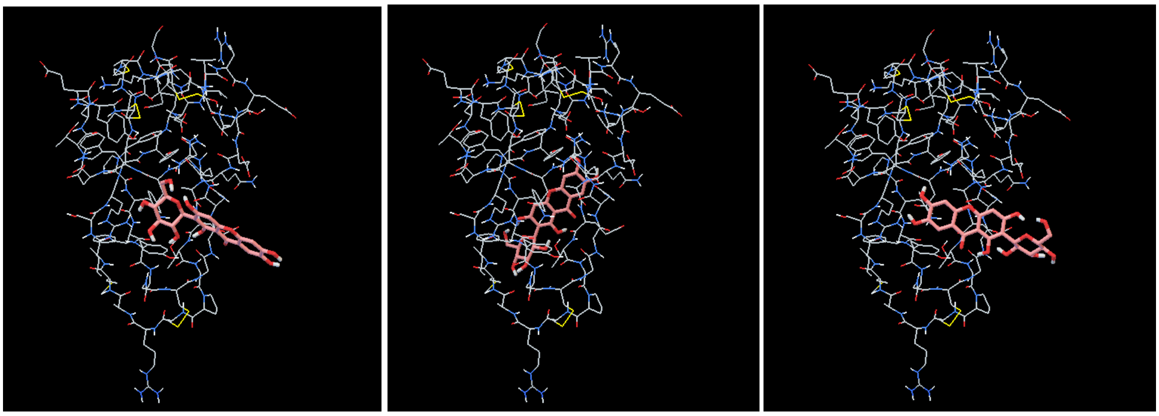
Figure 3: Most stable docked conformer ***(A)*** *showing the interaction of mangiferin with β-amyloid precursor protein receptor.*



(B) *(C)* *(D)*



(E) *(F)* *(G)*



(H) *(I)* *(J)*

Figure 4: Docked conformers ranked in order of stability

Table 1: Docking results of Mangiferin docked onto amyloid-β precursor protein receptor

Sl. No.	Run	*Conformer*	*Final Docked Value*	*Estimated Free Energy Binding (in kcal/mol)*	*Estimated Inhibition Constant, Ki (at 298.15K)*
1	6	A (most stable)	-12.421	-4.11	974.11μM
2	5	E	-12.513	-3.84	3.84mM
3	10	J	-11.223	-3.35	3.48mM
4	8	H	-9.661	-3.22	4.39mM
5	7	G	-11.637	-3.12	5.14mM
6	2	B	-12.109	-3.11	5.25mM
7	3	C	-11.739	-2.81	8.73mM
8	9	I	-10.978	-2.40	17.27mM
9	4	D	-10.797	-2.33	19.45mM
10	1	F	-10.727	-2.19	24.78mM

Number of distinct conformational clusters found: 9 out of 10 runs, using RMSD tolerance 2Å

Conclusion

In conclusion, the results of the present study demonstrates that the *in silico* molecular docking studies of mangiferin with amyloid-β precursor protein receptor exhibits binding interactions that are potentially responsible for the inhibition of the protease enzyme and further studies are needed for the development of a potential neuroprotective drug for the treatement of Alzheimer's disease.

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PRODUCTION OF PROTEIN HYDROLYSATES FROM FISH SKIN FOR DAIRY PRODUCTS

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Abstract: The aim of this research was studying the fish protein hydrolysates (FPH) produced from Salmon (*Salmo salar*) skin prepared by different methods. Four methods were used: enzymatic hydrolysis, acid hydrolysis, chemical (with acids or alkalis) and enzymatic hydrolysis with preserving agents upon the heating proposed to produce fish protein hydrolysates (FPH). All methods modified and adapted to optimized researches. All hydrolysates were vacuum freeze-dried and collagen content was determined in them. Depending on the collagen content in the FPH, methods may be ranked as follows: chemical method (with acids or alkali) (15,1%) < enzymatic hydrolysis (26,5%) < acid hydrolysis (30,0%) < enzymatic hydrolysis with preserving agents upon the heating (37,5%). The results showed that FPH prepared using enzymatic hydrolysis with preserving agents upon the heating had a greater amount of protein than FPH prepared using other methods. Citric acid preparation and sesame seeds covered the initial fish odour and off-flavours generated in fish hydrolysates. The sensory analysis of the final product allowed choosing the most rational content of components. Hydrolysis with preserving agents on the heating allowed obtaining collagen hydrolysate in dry form and without fish smell. This FPH may be used as a stabilizer in dairy products.

Keywords: fish protein hydrolysates, hydrolysis, fish odour, dairy products

Introduction

The fish raw material should be treated as a separate category among the sources of collagen. Collagen derived from hydrolysis has a number of advantages over its animal counterpart (Mintz *et al.*, 1991, Yang *et al.*, 2001, Dillow A. K. and Lowman A. M., 2002, Yamamoto *et al.*, 2014). Availability of raw materials is an important factor, the skin of marine and freshwater fish, and the rest raw material from the fisheries is widely used as a source of fish-derived collagen.

Fish collagen has a specific amino acid composition, with a high content of glycine, praline, and hydroxyproline. When ingested, collagen does not completely decompose to amino acids, which, combined with a small molecular weight and particle size, allows it to be absorbed through the walls of the intestine and spread throughout the body and bloodstream. Due to the high degree of identity of the biochemical composition of fish collagen to human collagen (up to 96%), cells can be stimulated to synthesize collagen in articular tissues, bones, skin dermis and other body systems (Bae *et al.*, 2008, Proksch *et al.*, 2014, Hashim *et al.*, 2015, Ramasamy and Ramadhar K, 2015).

Protein hydrolysates of fish-derived collagen have a great value. In the process of decomposition, collagen is broken down into individual peptides which have low molecular weight and as a result, penetrate through cell membranes faster, which allows classification of collagen-containing additives as functional components. During the transition to gluten and gelatine, collagen has the properties of dietary fibre, promoting metabolic processes, and also having a positive effect on the condition and functioning of the beneficial intestinal microflora (Silva *et al.*, 2014). Due to these properties, fish collagen hydrolysates show potential for the use in the composition of dairy products for normalization of microflora, restoration of cartilaginous and connective tissue and at the same time for giving the product the necessary structure (Shori, *et al.*, 2013).

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The limiting factor for an extensive use of fish protein hydrolysate is the presence of a strong fish odour. In this regard, the aim of this study was to make a comparative analysis of various methods of collagen hydrolysis currently used and the choice of the suitable method and optimum conditions for producing collagen hydrolysates for use in dairy technology.

Materials and methods

Skin of salmon fish which is belonging to the salmon family was used. The skin of the fish was washed, ground to a homogeneous state and divided into four parts for conducting hydrolysis using four methods. All methods were modified and adapted for the aims of the research, taking into account the specificity of dairy products.

Enzymatic Hydrolysis

Hydrolysis of fish skin was carried out under the action of proteolytic enzymes. The enzyme preparation: "Protepsin" was used for hydrolysis. The concentration of the enzyme preparation was 1:7 g / kg of raw material. Hydrolysis occurred in cheese whey medium at a temperature of 50 ± 2°C for 3 hours, which resulted in acceptable sensorial parameters of the finished product. Inactivation of the enzyme was performed by heating the solution to the boiling point. The product was then purified, degreased and clarified with a 1% solution of chitosan in 0.1 mol/l hydrochloric acid solution (raw material to chitosan solution weight ratio was 1:0.1). Further, the product was subjected to vacuum freeze-drying (Shironina, 2015).

Acid Hydrolysis

The skin of salmon, with scales and muscle tissue removed, was subjected to peroxide-alkaline treatment for two hours, with a component ratio of 7: 3. After that, the swollen and partially discoloured skin was placed in a 0.5% acetic acid solution for 72 hours. The resulting collagen dispersion was a translucent gel-like substance. It was then sent to freeze drying.

Chemical Hydrolysis (With Acids and Alkalis)

The method of complex processing (with acids and alkalis) of fish raw materials consisted of several successive stages of treatment with inorganic chemicals, during which alkaline and enzymatic hydrolysis occurs. The skin of Salmon scraped from scales and muscle fibre residues were crushed to a size of 2-3 mm and subjected to aqueous extraction at 40-45°C for 45 minutes at a feed- water ratio of 1: 1. The resulting solution was filtered, after which it was sent for treatment to obtain hyaluronic acid. The solid fraction was subjected to bleaching. 3% hydrogen peroxide was mixed with sodium chloride (20 g salt per 1L peroxide) and the raw material was steeped in the resulting solution for 12 hours. This was followed by filtration with separation of the solid fraction, which was placed in a 1.0-1.2% sodium hydroxide solution (raw material-to-solution weight ratio 1: 1) for 24 hours at 20-25°C. Once swelling was complete, the solution was neutralized with a 3% solution of boric acid. The swollen raw material was then proteolyzed with the enzyme «Protepsin» (0.5-0.6% of the solid fraction weight) for 1.5-2.0 hours at 37-40°C. The resulting product was rinsed with cold water and sent to freeze drying.

Hydrothermal Method

This method included the rinsing of raw fish material and heat treatment (80°C) in a cheese whey medium at a ratio of 1: 3 for 60 minutes. This was followed by the separation of the liquid phase, with the solid fraction placed in a clean whey solution and re-treated. The liquid phase was cooled and degreased by centrifugation. The isolation was carried out three times. Then the liquid phases were mixed, cooled and vacuum freeze dried.

Removing fishy Odour

A significant disadvantage of fish collagen hydrolysates is the presence of a specific fishy odour, which can adversely affect the flavour of the finished product. To disguise the specific fish odour, citric acid and sesame seeds were added in a mount of 1 to 5% to the raw material before freezing and freeze-drying

Determination of the Content of Collagen

The content of collagen in the resultant dried samples was determined using a modification of the Neuman and Logan method and expressed in the collagen content as % of the dry matter. The method is based on the isolation of hydroxyproline in the acid hydrolysis of the product sample, carrying out a colour reaction with the products of its oxidation and measuring the intensity of the developing colour. Spectrophotometer «Specord M 40» was used to measure the colour. The obtained data on hydroxyproline were reduced to a standard form for collagen-containing products (% of total protein content), using a special index of 7.63 (for collagen).

Preparation of Yoghurt

Yoghurt was prepared according to the following technology: skimmed milk powder was reconstituted with water at 45°C, then cooled to 10°C and held for 12 hours. The reconstituted skimmed milk was pasteurized at 83-85°C for 15 minutes and cooled to 42°C, then fermented. The composition of the starter included Thermophilic *Streptococcus* and *Lactobacillus bulgaricus*. Yoghurt samples were soured at 41±1°C until curd was obtained. Collagen hydrolysates in the amount of 1, 3 and 5% of the weight of reconstituted milk were introduced into the product in a dry form, before heat treatment. The yoghurt samples were then cooled to 4±2°C, viscosity values were evaluated and sensorial evaluation was determined.

Preparation of Sour Cream

Sour cream was prepared using the thermostatic method. Standardized cream with a fat content of 15% was homogenized at 60-85°C and a homogenization pressure of 12 MPa, then fish hydrolysate (as in yoghurt preparation) was added in a content of 1,3 and 5%, then pasteurized at 86±2°C for 2-10 minutes and cooled to a fermentation temperature of 32±2°C, fermented with a starter composed of *Streptococcus lactis* and thermophilic *Streptococcus*. The fermented cream was stirred, packed and allowed to ripen for 8 hours. The sour cream was allowed to ripen until curd was formed and acidity of at least 65°T was reached, then the sour cream was sent to the refrigerating chamber for cooling to 4±2°C and sour cream ripening.

Determination of the Viscosity of Dairy Products

The viscosity was measured using a low viscosity capillary type viscometer RHEOTEST 2. The shear stress values were measured at a strain rate from 0.167 to145.8 s-1. The temperature was always the same, equal to 20±1°C. The measurement results were mathematically processed in Table Curve® and Microsoft Office Excel using the Ostwald–de Ville model. The controls were yoghurt and sour cream samples prepared using traditional hydrolysate-free technology.

Sensory evaluation of prepared Dairy Products

Sensory evaluation of the products was carried out for the following parameters: colour, taste, smell, consistency and overall acceptance. The control was the traditional yoghurt and sour cream, prepared without fish hydrolysates. The panelists evaluated each attribute on a ten-point scale against traditional hydrolysate-free yoghurt and sour cream. Each panelist received individually about 15-20 ml of the control and other samples.

Statistical analysis

The results are presented as values ± standard deviation (SD). Tukey's test ($P < 0.05$) (Bower, 2013) was used to determine significant deviations between measurements. P-values below 0.05 were considered significant.

Results and Discussion

Hydrolysis is one of the ways to produce protein products from low-value raw materials, which allows obtaining preparations of isolated collagen proteins with high purity, as well as stimulation of the formation of the most important functional and technological properties in the context of the food industry branches, in particular, in the context of dairy products production.

After enzymatic hydrolysis, the resulting hydrolysate had a fibrous structure and a cream-white colour. Particle size was 1-2 mm (Figure 1.1). The hydrolysate formed a gel when adding water. The degree of hydration was 1:4.

Using the hydrothermal method, a fine powder with a colour ranging from white to cream-white with a particle size of up to 500 μm and a slightly fish odour was obtained (Figure 1.2).

The use of combined chemical hydrolysis (with acids and alkalis) allowed obtaining a hydrolysate in the form of a coarse white powder (Figure 1.3), which, when reconstituted with water, turned into a stable translucent dispersion, the degree of hydration was 1:5.

It was not possible to completely eliminate the reagents in the hydrolysate obtained by acid hydrolysis. So, it didn’t allow using this hydrolysate as a component in food products. In addition, this hydrolysate cannot be used in dairy products, since it does not dissolve in an aqueous medium.

Figure 1. Hydrolysates after vacuum freeze drying: 1 - hydrolysate obtained by enzymatic hydrolysis; 2 - hydrolysate obtained by hydrothermal method; 3 – hydrolysate obtained by chemical hydrolysis (with acids and alkalis).

The use of citric acid as a traditional means for disguising fish odour proved to be ineffective: no concentration of lemon preparation allowed reaching an acceptable level of disguising of fish odour. At a maximum dosage of 5%, a pronounced lemon smell, in combination with fish odour, produced an even more unpleasant effect. Sesame seeds served as a sorbent for aroma-forming compounds, allowing disguising fish odour. The rational content of added seed was 3% of the raw materials mass. With a greater content of the application, the aroma of sesame began to prevail, which was also extraneous and undesirable from the perspective of the experiment's aims.

Table 1 shows, those samples of hydrolysates after enzymatic and acid hydrolysis contained approximately the same content of hydroxyproline. The minimum content of hydroxyproline was isolated by chemical hydrolysis (with acids and alkalis). The highest content of collagen was found in the hydrolysate produced by the hydrothermal method from fish skin collagen. For this reason, this hydrolysate was selected for further researches.

Table 1 Hydroxyproline Content in Skin and Collagen Content calculated from the hydroxyproline values in the research hydrolysates. Hydroxyproline (OHPro) content in skin (g OHPro/100 g skin), collagen content calculated from the hydroxiproline values (g collagen/100 g hydrolysate)

Method	Hydroxyproline Content in Skin, %	Collagen Content (%)
Enzymatic Hydrolysis	3,47±0,16	26,5±0,03
Acid Hydrolysis	3,93±0,07	30±0,09
Chemical Hydrolysis (With Acids and Alkalis)	1,98±0,11	15,1±0,14
Hydrothermal Method	4,91±0,13	37,5±0,07

Collagen hydrolysate was added to yoghurt and sour cream, since for them one of the most important properties is thickness texture and resistance to flow or motion.

The results of the dependence of the shear stress on the shear rate of yoghurt and sour cream are shown in Figures 2 and Figure 3.

The data shows that for the researched products the force required to break the texture of the samples produced with collagen hydrolysate is higher than that in the control samples. The texture of the obtained hydrolysates, the increase in the content of dry components in dairy products, the binding and distribution of moisture in the texture of the protein gel framework contribute to the increase in the viscosity of the products. The use of collagen hydrolysates with a higher concentration (5%) leads to the production of sour-milk products with a more viscous consistency, while lower concentrations (from 1 to 3%) are advisable for production of drinking yoghurt.

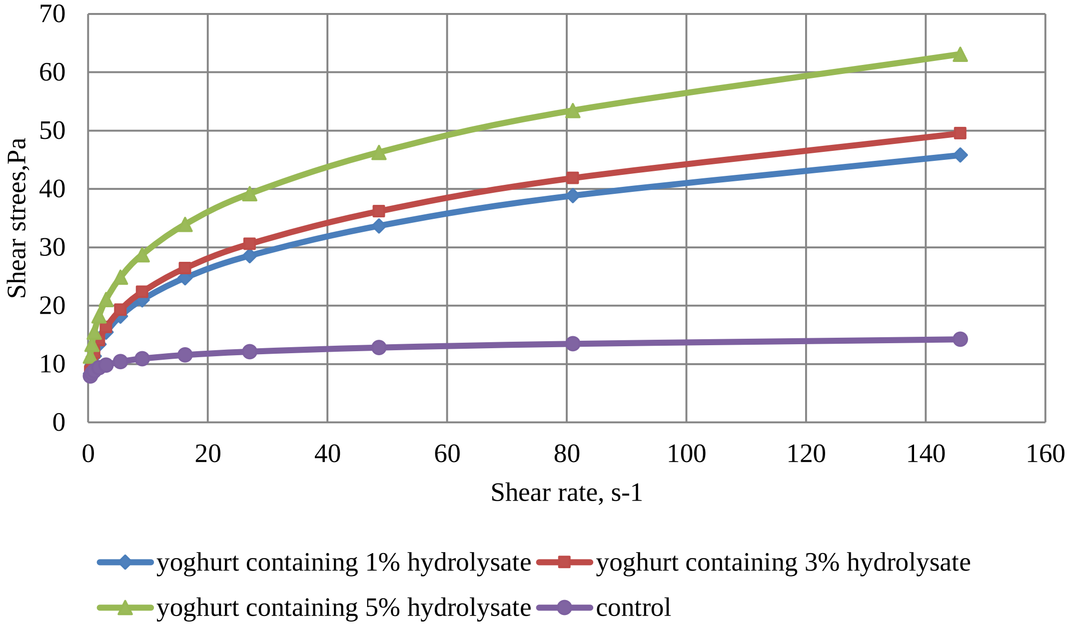

Figure 2. Dependence of shear stress on shear rate of yoghurt. Sample 1 - yoghurt containing 1% collagen hydrolysate, sample 2 - yoghurt containing 3% collagen hydrolysate, sample 3 - yoghurt containing 5% collagen hydrolysate, control - hydrolysate-free yoghurt

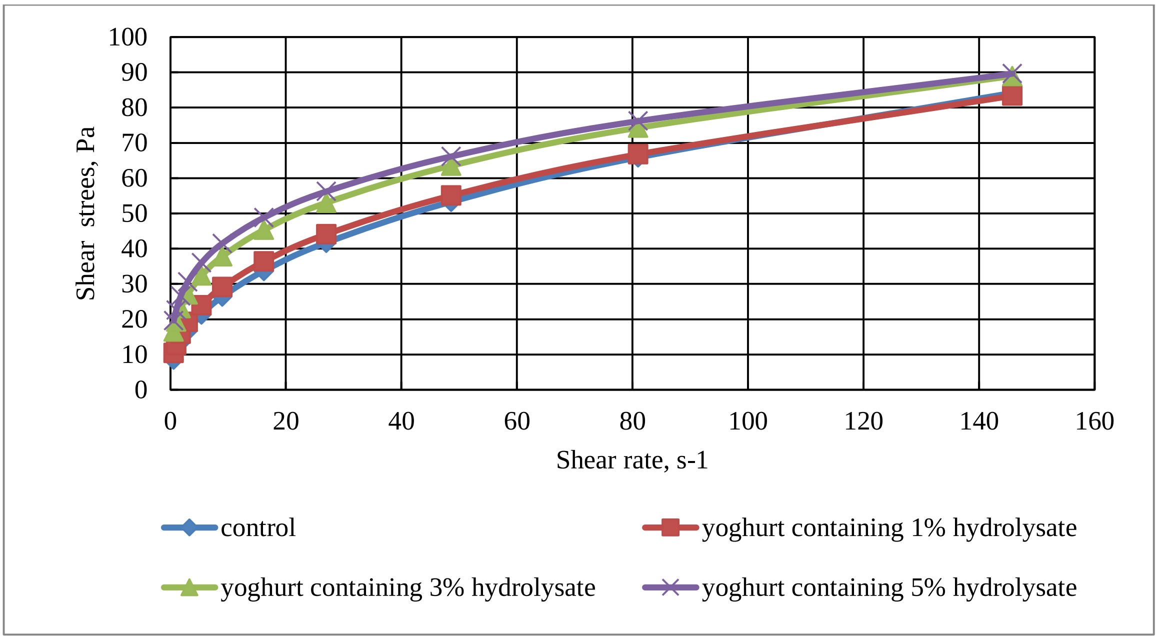

Figure 3. Dependence of shear stress on shear rate of sour cream: Sample 1 - sour cream containing 1% collagen hydrolysate, sample 2 - sour cream containing 3% collagen hydrolysate, sample 3 - sour cream containing 5% collagen hydrolysate, control - hydrolysate-free sour cream.

The sensorial properties of dairy products containing fish hydrolysates proved to be comparable to control samples produced by the traditional technology without hydrolysate. The addition of hydrolysate didn't affect the colour of the product. Also, what is very important, the yoghurt had a rich sour-milk taste without specific fish taste and smell. The panelists noted that the addition of 1 % hydrolysate had virtually no effect on the consistency of the yoghurt. The addition of 3 and 5% collagen hydrolysate led to yoghurt forming a thicker consistency. Panelists were noted in the yoghurt with the addition of 3% fish hydrolysate the best sensorial attributes. Yoghurt containing 5% fish hydrolysate has thicker structure, not characteristic of traditional yoghurt.

Similar data were obtained during the sensorial evaluation of sour cream produced with the addition of collagen hydrolysate. The panelists noted no fish taste and smell in the developed product. Collagen hydrolysate also had an effect on the consistency of sour cream, a thicker consistency was noted in the researched samples, which is confirmed by the data obtained in the researched of viscosity. In terms of the overall acceptance, the panelists gave preference to the sour cream with a content of collagen hydrolysate from 1 to 3%.

The results of the study revealed that the hydrothermal method is the most rational way of producing collagen hydrolysate for use in fermented dairy products. The use of fish hydrolysate in the production of yoghurt and sour cream improves the viscosity of dairy products, their consistency and sensorial properties.

Acknowledgements

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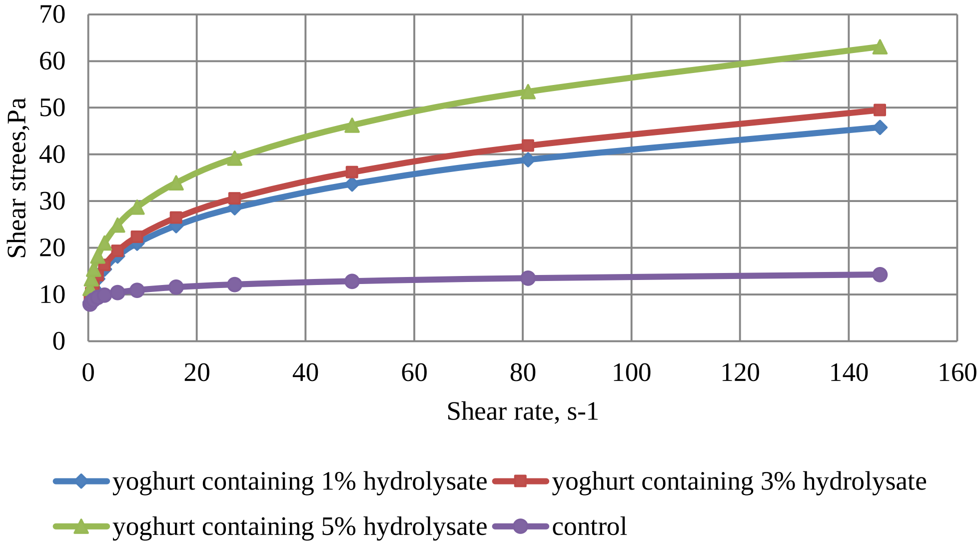
Appendix

Figure 1. Hydrolysates after vacuum freeze drying: 1 - hydrolysate obtained by enzymatic hydrolysis, 2 - hydrolysate obtained by chemical hydrolysis (with acids and alkalis), 3 - hydrolysate obtained by the method of natural structuring (hydrothermal method)

Table 1 Hydroxyproline Content in Skin and Collagen Content calculated from the hydroxyproline values in the research hydrolysates. Hydroxyproline (OHPro) content in skin (g OHPro/100 g skin), collagen content calculated from the hydroxiproline values (g collagen/100 g hydrolysate)

Method	Hydroxyproline Content in Skin, %	Collagen Content (%)
Enzymatic Hydrolysis	3,47±0,16	26,5±0,03
Acid Hydrolysis	3,93±0,07	30±0,09
Chemical Hydrolysis (With Acids and Alkalis)	1,98±0,11	15,1±0,14
Hydrothermal Method	4,91±0,13	37,5±0,07



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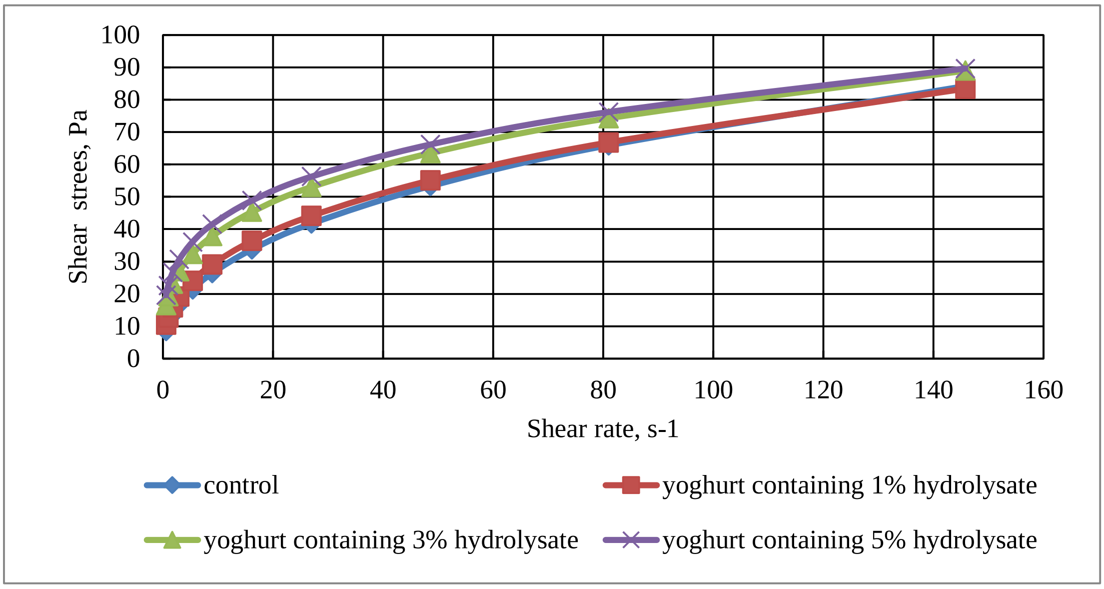

Figure 3. Dependence of shear stress on shear rate of sour cream: Sample 1 - sour cream containing 1% fish hydrolysate; sample 2 - sour cream containing 3% fish hydrolysate; sample 3 - sour cream containing 5% fish hydrolysate, control - hydrolysate-free sour cream.

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THE STUDY OF GENETIC VARIABILITY IN ASSOCIATION WITH ZINC UPTAKE EFFICIENCY IN *Triticum aestivum* L GENOTYPES

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Abstract: Diversity in agro ecosystems can improve the sustainability of cropping systems in terms of low external inputs and unpredictable climate changes. Agricultural ecosystems in the world are widely related to economical and social status. The major aim of agricultural ecosystems management is to maximize nutrient flow and human service materials. Nutrient efficiency in wheat is very complex. It includes nutrient acquisition efficiency and nutrient use efficiency. In this study 26 winter wheat genotypes were used to investigate the interactive effects between genotypes and the use efficiencies of the Zn micronutrient of the grain. An experiment was carried out in Agricultural and Natural Resources Research and Education, Center of West Azerbaijan, in which Genotypes were planted in complete randomized block design with three replications. The traits such as plant height, biological and grain yield, harvest index, length of spike, number of kernel per spike, thousand kernel weight and spike weight were measured or calculated. Concentration of Zn in the grain was measured by dry combustion using Perkin Elmer 2380 Atomic Absorption Spectroscopy. Zinc uptake was calculated by multiplying grain Zn concentration in the grain yield. It was revealed that biomass weight of whole plot, plant height, number of kernel per spike, grain yield of main culms, biological yield, length of spike and number of spikelet/spike were positively correlated with grain yield, while the grain protein percentage was negatively correlated with the grain yield. Variance analysis showed that there were highly significant differences among traits. The results obtained in this study indicate that nutrient use efficiency of the Zn varies widely within wheat genotypes. Some genotypes were identified as being Zn use efficiency. These are considered as low-input genotypes. It seems that a special breeding programmer of crop cultivars for low Zn nutrient and stress condition could be successful. Improving the cultivar response to Zn nutrient will help to reduce inputs and hence protect the environment.

Keywords: Wheat, Zn use efficiency, Genetic variability, low input

Introduction

Mineral elements play essential roles in biochemical and physiological functions of any biological system. In plants, appropriate mineral availability is necessary to every aspects of development including seed germination, seedling development (Welch, 1999), yield formation as well as mineral deposition in grain (Yilmaz et al., 1998; Welch, 1999).Deficiencies of elements such as zinc are well known in all cereals and cereal-growing countries. Increasing the quality of products in order to prevent malnutrition and some diseases caused by nutrient deficiencies, especially iron and zinc is very important. Achieving sustainable agricultural goals, it is possible to improve the quantity and quality of products via producing cultivars with desirable genetic traits. These cultivars are resistant to nutrient deficiencies and have high efficiency in nutrient uptakes (Hasanzadeh Gorttapeh and Mozafari, 2004).

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Wheat (*Triticum* spp.) is the major staple food crop in different parts of the world. It is cultivated in about half of areas in developing countries such as the Middle East, central India and the Mediterranean region of west Asia and the other countries including Ethiopia, Argentina, Chile, Russia, the United State, Italy, Spain and Canada. Generally, wheat production is low in developing countries due to usage low level inputs (e.g., fertilizer, water) in semi- arid regions. In addition, yields may be reduced by insects attacks, poor crop management and deficient weed control (Connor, 2011).Considering the high geographic diversity in different regions, it is necessary to evaluate the cultivars with different characteristics; then these crops should be applied in breeding programs to prevent the yield reduction and to raise nutrients uptake efficiency (Haneklaus and Schnug, 1993; Hassanzadeh Gorttapehand Salehzadehi, 2010).Yields of crops are often limited by low levels of mineral micronutrients in soil such as zinc (Zn), especially in calcareous soils of arid and semiarid regions. Zinc deficiency in most agricultural soils of the country is a neglected point in production chain. High-yielding wheat varieties should be planted in fertile soils. They have lower yields than their potential due to lack of nutrients including Zinc. Since some of the nutrients may be lost or become inaccessible in various ways. Achieving the optimal yield per unit is possible by applying chemical fertilizers (Dambroth and Bassam. 1990).

The results of recent researches in different parts of the country have proven the effective role of this vital element in increasing the wheat quantity (Malkoti, 2001). Results relating to zinc fertilizers use, showed the low absorption of this element (less than 1%) and different ability of cultivars in its absorption (Pecetti et al., 1992.). Studying the absorption of nutrients including Mg, Ca, K, P, N and Zn in 15 wheat cultivars, Sarik et al. (1990) found a high genetic diversity among wheat cultivars in terms of nutrientusage efficiency while some cultivars are more tolerant to its deficiency. Smith (1934) stated that the absorption of nutrients by the plant is influenced by genetic traits.

Table 1 - Physico-chemical characteristics of the experimental site (Dr. Nakhjivani Research Station)

Cu	Zn	Mn	Fe	Available K	Available P	Clay	Silt	Sand	Total N	Organic C	Neutralizing	Total acidity saturation	Electrical conductivity	Saturation percent	Depth	Sample View
Mg per Kg						Percent						)pH(	)ds/m(	)s.p(	)cm(	
0.66	0.54	0.7	4.7	280	3.1	9	8	83	0.07	0.65	2.5	8	0.67	25	30 - 0	Composite sample

Breeding programs had a significant importance in terms of nutrient usage efficiency, plant characteristics, soil type and climatic factors. Haneklaus and Schnug (1993) observed a remarkable difference in the nutrients absorption, especially zinc, manganese and phosphorus in different wheat cultivars during their experiments. Investigating the different wheat varieties response to low-energy elements, Takar (1991) stated that deficiency of low-energy elements reduced the production potential in many soils of India. However, resistant cultivars application has been an effective step towards increasing products per unit area. Regarding demands for food, attempt should be focused in improving the quality and quantity of this product. Therefore, it is possible to assess desirable cultivars by identifying local lines with high efficiency of nutrient uptake, classifying favorable traits and determining the correlation between desirable traits and using them in breeding programs to correct high-yielding wheat cultivars and commercial cultivars.

The aim of this study was to introduce wheat varieties with high zinc uptake efficiency for cultivating and using in breeding programs. The results of this paper can be remarkable due to the limited sources of zinc and its deficiency in Iranian soil (Malkoti, 2001).

Material and method

Experiment was conducted at Research Station of Dr. Nakhjivani, West Azerbaijan Agricultural and Natural Resources Research and Esucation Center, Urmia, Iran. (Latitude 37º53'N and longitude 45º10'E and altitude 1325m) during the 2010 and 2011.The annual rainfall ranged from 300mm to 350mm. The mean annual temperature was around 13.1°C. The coldest and hottest months of year were December and July, respectively. The results showed that the irrigation water had a very low salinity and sodium which was in class c2s1 based on Will Cox classification. The site soil was non-saline, sandy loam with a small amount of lime and pH 8. Phosphorus content was too low.

The field has been prepared in fall. After performing physic-chemical analysis (Table 1), required chemical fertilizers were distributed uniformly at the field. The experimental design was a split plot in a randomized complete block design with three replications. The experiment was consisted of seeds 23 selected lines and 3 common varieties (Alamut, Zarin and Shahryar). The first factor was consisted of three treatments: control (without fertilizer), consuming NPK, NPK+Zn. The second factor was Seeds of lines and wheat cultivars. All crop managements were carried out and weeds were controlled with application of 2, 4-D (1.5 L/ha) in tiller stage. Traits such as biological and grain yield, harvest index and 1000 grain weight were measured at the end of the experiment. Zinc concentration was measured for the grain and zinc yield was calculated according to formula (1)

Zinc yield=zinc concentration × grain yield/ha (1)

Zinc absorption efficiency was calculated from the formula (2):

$$\text{Zinc absorption efficiency} = \frac{\text{(Zn uptake in the fertilizer plot - Zn uptake in control plots)}}{\text{The amount of Zn fertilizer applied}} \quad (2)$$

Stress susceptibility index (SSI) was Calculated from formula (3)

S=1- (Ydi/ Ypi)/ 1-D (3)

D= YD/YP

Ydi=Mean yield under Zn deficiency (control without fertilizer)

Ypi=Mean yield after applying Zn fertilizer

YD=Mean yield of all lines under Zn deficiency (control without fertilizer),

YP= Mean yield of all lines after applying Zn fertilizer (non-stress)

All data were statistically analyzed by the means of variance analysis followed by Duncan's multiple test. Simple correlations between traits were calculated and local lines were classified according to different characteristics and also environmental sensitivity index.

Results and Discussion

The results of analysis of variance are presented in Table 3. Based on the table, fertilizer and genotype effect were statistically significant on seed yield, biological yield, harvest index, 1000 grain weight, zinc concentration in seed and zinc yield per hectare, in probability level of 1%.

Mean Comparison of traits in fertilizer treatments is presented in Table 4. Maximum grain yield, biological yield, harvest index and 1000 grain weight were achieved from NPK+Zn fertilizers treatment due to increasing nutrients uptake and photosynthesis rate. Moreover, Zn concentration in grain and its adsorption per unit area were highest.

Table 2- Number, code and collected site of lines and varieties

Collected site	Identification Code	Genotype number	Collected site	Identification Code	Genotype number
Ardabil	Kc-330	14	East Azerbaijan	Kc-20	1
Ardabil	Kc-2143	15	East Azerbaijan	Kc-30	2
Ardabil	Kc-2147	16	East Azerbaijan	Kc-40	3
Ardabil	Kc-2149	17	East Azerbaijan	Kc-44	4
Ardabil	Kc-2151	18	East Azerbaijan	Kc-50	5
Ardabil	Kc-2155	19	East Azerbaijan	Kc-58	6
Khorasan	Kc-3079	20	West Azerbaijan	Kc-113	7
Khorasan	Kc-3095	21	West Azerbaijan	Kc-132	8
Khorasan	Kc-1717	22	West Azerbaijan	Kc-145	9
Khorasan	Kc-1773	23	West Azerbaijan	Kc-1974	10
West Azerbaijan	Alamut	24	Zanjan	Kc-4144	11
West Azerbaijan	Zarin	25	Kurdistan	Kc-4173	12
West Azerbaijan	Shahriyar	26	Kurdistan	Kc-4175	13

Mean comparison of genotype on grain yield, biological yield, harvest index, 1000 seed weight, zinc concentration in seed and its yield are presented in Table 5. Maximum grain yield (6.9 ton/ha) was obtained from Shahriar cultivar, followed by Zarrin, East Azarbaijan (line 2), Kurdistan (genotype 12), Zanjan (genotype 11) and Ardebil (genotype 15) with4.09, 4.07, 3.99, 3.92 and 3.72 tons/h, respectively while the lowest grain yield (2.5 t/h) was achieved from khorasan (genotype 23) cultivar.

The biological yield trend was similar to grain yield. Shahriar cultivar produced the highest yield among the studied genotypes. Zarrin and genotypes (2, 12 and 11) had the most biological yield, respectively. Biological yield in Ardebil and Khorasan was the lowest (Table 5).

Harvest index is an important characteristic that indicates the ratio of grain yield to biological yield. According to experiments, Selection based on harvest index increases grain yield and fertilization in plants (Viedt and Spanakakis, 1989.).

The highest 1000-seed weight was obtained from Shahriar and Zanjan (genotype 11), respectively which was significantly higher than the others (Table 5). This trait is one of the most important components of grain yield and its increase can improve the grain yield. The Zinc concentration in grain was significantly higher in genotypes 20 and 21 than the others.

This trait had the lowest amount in genotypes 1, 9 and 13, respectively. It showed diversity among genotypes in terms of Zn absorption and its storage. Genotypes with high ability for storing are more important. They have high quality in the food chain.

Mean comparison of Zn Yield (Zn concentration × grain yield) is shown in Table 5. The highest yield of zinc (31.8 kg ha-1) was produced in Shahriar cultivar due to its higher grain yield. This characteristic was lowest in genotypes (3, 23), respectively due to less grain yield or zinc adsorption per unit area. Regarding the results, wheat can absorb (122 to 318 g/h) zinc. Therefore, this element should be added to increase the soil fertility.

Table 3- Analysis of variance between Wheat cultivars with fertilizer treatments

Zn absorption (mg/ha)	Zn content (ppm)	1000 grain weight (gr)	Harvest index (%)	Biological yield (kg/ha)	Grain yield (kg/ha)	df	Sources
**33.38	169110216.1	**14.52	0.30	196772.8	289396.2	2	Block
**318.74	**198635112419.4	**680.27	**1924.82	**196584845.8	**84016702.3	2	Fertilizer
0.153	705619004.7	0.305	2.152	121468.2	371474.3	4	(I) Error
**122.11	**12465390911.6	**136.63	**6.855	**38191391.91	**5969603.1	25	Cultivar
14.69	151192446.6	1.23	0.494	257055.3	274954.1	50	Cultivar× Fertilizer
2.91	439199139.6	1.91	1.405	209167.87	208561.8	150	(п) Error
3.91	13.17	3.51	2.58	6.08	13.1		C.V

** means Significance in probability level of 1%

The analyses of variance about zinc efficiency are presented in Table 6. Based on the table, genotype effect on zinc adsorption was statistically significant at 1%. Mean Comparison of data showed that the zinc -use efficiency was higher in West Azarbaijan (genotype 10) among the others.

Table 4- mean Comparison of studied traits in fertilizer treatments

Zn absorption mg/ha) (	Zn content) ppm (	1000 grain weight) gr (	Harvest index (%)	Biological yield kg/ha) (	Grain yield (kg/ha)	treatment
45.70B	105356C	36.08C	40.23C	5741.32C	2308.7C	No Fertilizer
43.89C	166680B	40.19B	48.13B	8042.99B	3810.7B	Consuming NPK
48.01A	205440A	41.80A	49.39A	8786.29A	4299.1A	consuming NPK + Zn

Similar letters indicates no significant difference in probability level of 5% in each column.

Table 5 mean Comparison of studied traits in wheat genotype

Zn absorption mg/ha)(	Zn content Mg/kg)(	1000grain weight)gr(	Harvest index)%(	Biological yield kg/ha)(	Grain yield (kg/ha)	Genotype
133045IJ	36.21Y	34.6K	46.48ABCDE	7405.3DEFG	3501.5 CDE	1
193915B	46.85K	36.98HI	46.18BCDEF	8749.1B	4074.1 B	2
12239J	43.12U	35.28JK	45.27EFGH	6128KL	2818.7GH	3
135466IJ	43.35S	37.49GHI	45.90DEFG	6688.3HIJ	3126.2EFG	4
137924HIJ	47.25J	38.15GH	44.97FGH	6332.3JK	2890.4FGH	5
151870DEFGHI	49.62C	43.33C	45.55DEFG	6776.9HIJ	3135.8EFG	6
160642CDEFGH	47.39I	42.76CD	46.39ABCDE	7140.6EFGH	3376.4DEF	7
164197CDEFGH	45.10N	41.28EF	45.91CDEF	7566.6DE	3522.7CDE	8
149789EFGHI	42.75W	43.36C	47.06ABC	7076.7FGH	3394.4DEF	9
168166CDEF	48.85E	38.51G	45.52DEFG	7447.9DEF	3446.3CDE	10
176972BC	44.22Q	46.07B	44.93FGH	8596.3B	3928.3BC	11
172959BCD	43.93R	43.75C	45.43EFG	8712.1B	3997.0B	12
142022GHIJ	40.25X	41.72DEF	45.59DEFG	7431.8DEF	3444.1CDE	13
1551143DEFGHI	43.33T	37.02HI	47.01ABC	7308.5EFG	3494.4CDE	14
170778CDE	45.42M	42.61CD	45.59DEFG	8038.8C	3718.1BCD	15
146167FGHI	48.28G	37.55GHI	45.44EFG	6549.9IJK	3021.7EFGH	16
131461IJ	45.10N	34.89K	44.45GH	6676.6HIJ	3007.1EFGH	17
139441HIJ	44.59P	34.72K	46.03CDEF	6570.0IJK	3068.4EFGH	18
147619EFGHI	44.89O	36.95HI	46.97ABC	6426.0JK	3189.8EFG	19
138164HIJ	54.89A	37.08GHI	47.65A	5686.8L	2764.4GH	20
170854CDE	51.94B	36.29IJ	47.36AB	6948.7GHI	3352.2DEF	21
166666CDEF	48.72F	36.39IJ	46.82ABCD	7797.3CD	3371.2DEF	22
122232J	47.77H	36.48IJ	44.14H	5708.8L	2577.5H	23
149575EFGHI	49.48D	40.86F	45.93CDEF	6408.3JK	3020.5EFGH	24
176533BC	42.75V	40.73F	26.01CDEF	8800.9B	4097.9B	25
318132A	46.52L	48.44A	45.33EFGH	16632.4A	6946.9A	26

Similar letters indicates no significant difference in probability level of 5% in each column.

Table 6- Mean comparison of Zn- use efficiency and stress susceptibility index in different genotypes at 5%

MS			
SSI	Zn- use efficiency	df	Sources
1.7518	0.1702	2	Replication
1.8865	0.4464**	25	Cultivar
1.1756	0.10570	50	Error
3.60	15.84		CV

means Significance in probability level of 1% **

Statistically, this genotype didn't have any difference with most of genotypes except 6, 13, 18, 21 and 25. Khorasan cultivar (genotype 21) had the lowest zinc efficiency (Table 7). Researchers mentioned the effects of genotype and environment, including climatic and soil conditions, as effective factors in the absorption and storage of nutrients (Sorm, 1984).

Table 7- mean comparison of Zn- use efficiency in different genotypes at 5%

Zn- use efficiency	Genotype	Zn- use efficiency	Genotype
0.7343ABCDE	14	1.0177AB	1
0.6950ABCDE	15	0.9542AB	2
0.7585ABCD	16	0.5985ABCDE	3
0.1873DEF	17	0.8326ABC	4
0.7518ABCD	18	0.8169ABCD	5
1.0292AB	19	0.4353BCDEF	6
0.1116F	20	1.0411AB	7
0.7675ABCD	21	0.9284AB	8
0.9530AB	22	1.087A	9
0.2305CDEF	23	0.5958ABCDE	10
0.4932ABCDEF	24	0.7188ABCDE	11
0.6302ABCDE	25	0.1057FG	12
1.025AB	26	0.8491ABC	13

Investigating of different genotypes based on stress susceptibility index (Fisher and Maurer, 1984)

The genotypes were classified based on stress susceptibility index(Fisher and Maurer,1984).Based on this coefficient, since the difference of genotype yields under stress conditions is less than normal condition or the obtained number is closer to 1, this genotype is more resistant to environmental stress. According to the results of this study, genotypes were classified in three groups: resistant (1-3), semi-sensitive (3-6) and sensitive (more than 6). The numbers and classifications of each group are presented in Table 9. Khorasan cultivar (genotype No. 22) was the most sensitive to zinc deficiency. Zarrin and Shahriar were more resistant than other genotypes and the remaining genotypes were placed in semi-sensitive group. Correlation coefficients between the measured traits are presented in Table 9. Grain yield had a significant and positive correlation with biological yield and 1000 grain weight and their increasing can improve grain yield. The zinc use efficiency and its concentration in grain, grain yield and zinc yield had positive and significant correlation ($p<0.95$), which meant improve zinc use efficiency by increasing each of them.

Increasing quality of food along with preserving the environment is one of the main goals in today’s societies. In this regard, increasing soil fertility and preventing nutrients losing plays an important role in improving the quantity and quality of products. However, the quality of soil and environment should be protected in sustainable agriculture. Different factors have significant impacts on soil fertility such as type of cultivated product and the previous product, planting date (spring or autumn), amount of nutrients in soil, physical characteristics of the soil (texture and drainage), the purpose of planting (forage, grain, or dual purpose) and genotype type (7).

Table 8- classifying genotypes based on stress- susceptibility index (Fisher and Maurer, 1984)

Class	SSI	Genotype	Class	SSI	Genotype
Semi-sensitive	3.65	14	Semi-sensitive	3.46	1
Semi-sensitive	3.50	15	Semi-sensitive	3.80	2
Semi-sensitive	3.68	16	Semi-sensitive	3.53	3
Semi-sensitive	3.58	17	Semi-sensitive	3.49	4
Semi-sensitive	3.57	18	Semi-sensitive	3.49	5
Semi-sensitive	3.92	19	Semi-sensitive	3.56	6
Semi-sensitive	3.73	20	Semi-sensitive	3.56	7
Semi-sensitive	3.59	21	Semi-sensitive	3.57	8
sensitive	6.50	22	Semi-sensitive	3.86	9
Semi-sensitive	3.43	23	Semi-sensitive	3.36	10
Semi-sensitive	3.52	24	Semi-sensitive	3.68	11
Resistant	2.38	25	Semi-sensitive	3.70	12
Resistant	2.54	26	Semi-sensitive	3.478	13

The genotype is one of the main factors influences the products. Genotypes with ability of high nutrients absorption and storing them in their organs can enhance the quality of product and prevent malnutrition or some nutritional deficiency diseases. Moreover, cultivation of these genotypes can lead to less fertilizer use. Zn element has a significant role in raising production and quality improvement among the nutrients. Based on results of the paper, there was a high diversity in grain yield, biological yield, harvest index, Zn absorption and its yield among genotypes (Table 8). Genetic potential of genotypes is a determinant of environmental factors in optimal use and genotypes 2, 11, 12, 25 and 26 were the best in this regard. Some researchers also suggested that the yield potential varies among cultivars and local lines. The absorption and storage of nutrients were different among them (1, 2, 3, 4, 5, 7 and 10).Zinc- uptake efficiency differed among genotypes and those which absorbed more Zn element were more efficient (1, 2, 7, 8, 9, 19, 22 and 26). According to experiments, genotypes that responded to low levels of nutrients could be used to improve crops and produce high potential cultivars (9, 17, 21 and 19). Improving wheat cultivars in order to absorb more elements can increase the efficiency of low-energy elements adsorption in soil.

Table 9: correlation coefficient between wheat lines and cultivars in measured traits

traits	1	2	3	4	5	6	7	8
1. grain yield	1							
2. Biological yield	**0.993	1						
3. Harvest index	-0.053	0.121-	1					
4. 1000 grain weight	**0.637	**0.624	0.175-	1				
5. Zn content	0.147-	0.119-	0.165	0.018-	1			
6. Zn absorption	**0.962	**0.962	0.014-	**0.633	0.109	1		
7. Zn- use efficiency	*0.321	*0.289	*0.349	0.124	*0.362	*0.294	1	
8. SSI	*0.319-	*-0.285	*0.304	*0.296-	0.187	0.239-	0.133	1

**and* means significance at 1% and 5%, respectively

Finally, based on cluster method, treatments were placed in three main groups.

Samples with high seed yield, high Zn use efficiency and low susceptibility to its deficiency: shahriyar cultivar.

Samples with fairly high seed yield, fairly Zn use efficiency: 7, 21, 26, 42, 53, 56, 71, 89, and 94: Zarrin cultivar.

Samples with low seed yield, low Zn use efficiency: 4, 10, 12, 16, 20, 33, 68, 13, 74, 76, 77, 80, 83 and 100: Almut clutivarl.

```
C A S E    0         5        10        15        20        25

  Label  Num  +---------+---------+---------+---------+---------+

          5   ⇩⇘

         20   ⇩▫

         18   ⇩▫

          4   ⇩▫

         13   ⇩▫

          1   ⇩▫

         17   ⇩▫

          6   ⇩▫

         14   ⇩▫

         16   ⇩⇳⇩⇘

         24   ⇩▫⇔

          9   ⇩▫⇔

         19   ⇩▫⇔

          3   ⇩▫⇔
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          23   ⇩⬀⇔

          11
⇩⬂▫⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⬂

          25   ⇩▫⇔⇔

          15   ⇩▫⇔⇔

          21   ⇩▫⇔⇔

          12   ⇩▫⇔⇔

          10   ⇩▫⇔⇔

          22   ⇩⇳⇩⬀⇔

           8   ⇩▫⇔

           7   ⇩▫⇔

           2   ⇩⬀⇔

          26
⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⬀
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Table 3- Dendrogram of the studied treatments

Sarik and Momsilovich (16) reported increasing fertilizer use efficiency by examining the nitrogen uptake efficiency among wheat genotypes. The zinc element investigation in stress susceptibility index showed that genotypes 25 and 26 were highly resistant and genotype 22 was very sensitive (Table 11). The resistant genotypes can be used in breeding programs.

Conclusion

Increasing the quality of food along with preserving the environment damage is among the main goals in sustainable agriculture. It has been proven that increasing soil fertility and preventing the nutrients loss lead to improve quality and quantity of products. Distinct factors impact on soil fertility including type of crop(forage, grain or both of them), previous crop, cultivation date, nutrients content in soil and type of genotype. Genotype is the main factor in improving the quality and quantity of products.

Based on this paper, there was a great diversity in grain yield, biological yield, harvest index, Zn absorption and its yield (table 9). Also, the genetic potential was the most important factor in optimal use.Zarrin and Shahriyar cultivars were the best in condition of samples No. 7, 53 and 56. Some researchers stated that yield potential is different among cultivars and local lines. Moreover, absorption and storage of nutrients are various (Altin and Frey, 1990; Dambroth and El Bassam, 1990; Damisch and wilberg, 1991; Fichbeck, 1988; Haneklaus and Schnug, 1993; hassanzadeh-Gorttapeh, 2007) as well as Znuse efficiency. Samples with higher ability toabsorb nutrient (Zn) had the highest efficiency (genotypes 4, 7, 10, 26, 33, 80, 94 and Shahriyar cultivar).

Superior genotypes or genotypes which react to low levels of nutrients can be used in breeding programs. As a result, high potential lines can be produced while increasing the efficiency of low-input elements. Saric and Momcilovic (2004) showed increasing the efficiency of fertilizer application by examining the Nuse efficiency in superior lines. Investigating SSI indicated that Zarrin and Shahriyar cultivars were very tolerant and genotype

94 was sensitive. Resistant and sensitive cultivars have a great importance in breeding activities. Takkar (2001) stated that superior genotypes should be considered and produced in order to prevent malnutrition.

According to this paper, it seems that it is possible to modify and produce cultivars to increase the quality of production in low nutrient conditions. Appling this method, we can significantly reduce consumption of fertilizer in agriculture and prevent the environment damage. Furthermore, it is possible to select genotypes with high efficiency in nutrients absorption and storage (i.e. zinc) using modern technologies and biotechnologies such as RAPDs, RFLPs and PCRs due to genetic variation among genotypes.

Suggestions

1-Considering the different susceptibility of wheat genotypes to zinc element, it is recommended to pay attention to the specific sensitivity of plants at the time of fertilizer consuming.

2- It is suggested to investigate uperior genotypes for absorbing different amounts of fertilizer and obtaining accurate results about selection of efficient cultivars.

3- It is also important to study the selection of best genotypes in order to reduce the inputs (fertilizers, water, etc.) per unit area

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