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Stimulation of the Gene and Protein Expression of Chondrocytes Seeded in Chitosan Scaffolds by Ultrasound

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In this study, a low-intensity diffuse ultrasound (LIDUS) signals at 5.0 MHz (0.14 mW cm^{-2}) was employed to stimulate bovine chondrocytes seeded in 3D chitosan-based matrices. While the duration of application was constant at 51 sec, US was applied 1-time, 2-times, 4-times and 8-times per day, and the impact of US on the biosynthetic activity of chondrocytes and the expression of chondrocyte-specific genes were evaluated. When stimulated with continuous US for predetermined time intervals, chondrocytes had higher levels of type-II collagen, aggrecan, L-Sox5, and Sox9 mRNA expression when compared to controls, however, under the same conditions, the expression of MMP-3 was down regulated. Interestingly, both Sox5 and Sox9 genes coordinately responded to changes in US stimulation and generally mirrored the response of collagen type-II transcript to changes in US stimulation. RT-PCR analysis revealed that US stimulation increased the gene expression of cell-surface integrins α5 and β1. The expression of integrins α2 was down regulated by US treatment, suggesting that multiple integrin subunits may be involved in the regulation of chondrocytic function in response to US stimuli. The enhancement in the abundance of the mRNA transcripts upon US stimulation was observed to correlate with the protein expression of collagen type-I, collagen type-II, and integrins α5 and β1. In conclusion, US stimulation regimen employed was shown to modulate the proliferative capacity, biosynthetic activity, and integrin mRNA expression of articular chondrocytes maintained in 3D matrices.

Keywords: *ultrasound, gene expression, protein expression, collagen I and II, Sox factors*

INTRODUCTION

Articular cartilage is essentially avascular, alymphatic, and aneural and the chondrocytes that populate the extracellular matrix (ECM) are strain-sensitive cells that possess the ability to sense mechanical stimulation and further modulate the cellular metabolism and phenotype. Ultrasound treatment has emerged as one of the alternatives to facilitate cartilage repair and restoration (Wang et al., 2005; Naito et al., 2010) and in addition, studies in the last ten years have shown that chondrocytes maintained in *in vitro* culture can be stimulated by ultrasound (Parvizi et al., 1999; Hsu et al., 2007). Several studies have addressed the effects of low-intensity pulsed US on cartilage at the cellular level (Ebisawa et al., 2004; Parvizi et al., 1999) and interestingly, the response to US stimulation (1.0-MHz signals with spatial and temporal average intensities of 50 or 120 mW cm^{-2}) was found to differ between a monolayer culture and cells maintained in alginate or collagen hydrogels (Parvizi et al., 1999). In this study, we characterize the response of chondrocytes to an US stimulation regimen (5.0 MHz, 51-secs/application), both at the gene and the protein level. We hypothesize that (1) ultrasound regulates the gene and the protein expression of aggrecan and collagen and (2) that transcription factors Sox-9 and Sox-5 are involved in the upregulation of collagen-II under US stimulation. In parallel, we also evaluate the effect of US stimulation on the expression of integrins, namely α5 and β1, as they have been implicated in mechanotransduction in many cell types, including chondrocytes, and on the gene expression of the matrix remodeling genes, MMP-3 and COX-2.

MATERIALS AND METHODS

Isolation of chondrocytes. Articular cartilage tissue was obtained from the shoulder joints of freshly slaughtered 6-monthold calves from a local abattoir and chondrocytes were isolated as detailed elsewhere (Noriega et al., 2007). Discarded tissue from the shoulder joints of 6-month old calves were obtained from a local abattoir and chondrocytes were isolated using a protocol detailed elsewhere.

Scaffold preparation, cell seeding ultrasound

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(US) stimulation. Scaffold preparation, cell seeding ultrasound (US) stimulation were performed as described by Hasanova et al. (2010).

Cell count and viability. Cell count and viability performed as described elsewhere (Noriega et al., 2007).

mRNA gene expression analysis. Total RNA was isolated from cells using trizol reagent and gene RT-PCR analysis was performed using primers as described by Hasanova et al. (2007, 2010).

Western blotting analysis. Western blotting analyses were performed as described by Hasanova et al. (2010).

Statistical analysis. For statistical analyses Student's t -test was used for statistical analysis and statistical differences were declared as p<0.01.

RESULTS

Cells that were stimulated 4 times a day (4×) showed a significant increase in viability as compared to control (data not shown). The application of US was observed to impact the cell number obtained and when compared to control higher cell numbers were obtained when US was applied 8-times/day (data not shown). No statistical difference in the cell count was observed between 4 applications of US/day and 8 applications of US/day. The impact of US stimulation on the mRNA expression of chondrocytic markers (collagen-II, collagen-I, aggrecan) was examined by RT-PCR as a function of the frequency of US application and normalized with respect to GAPDH (Figure 1A). Compared to the control, higher levels of collagen-II and collagen-I mRNA were observed when cells were stimulated with US at four-times and eight-times per day, however, the mRNA expression of aggrecan mRNA was not significantly impacted by US stimulation. The only transcription factor that is required for chondrogenesis is Sox-9, which directly regulates the transcription of the collagen II and aggrecan genes in

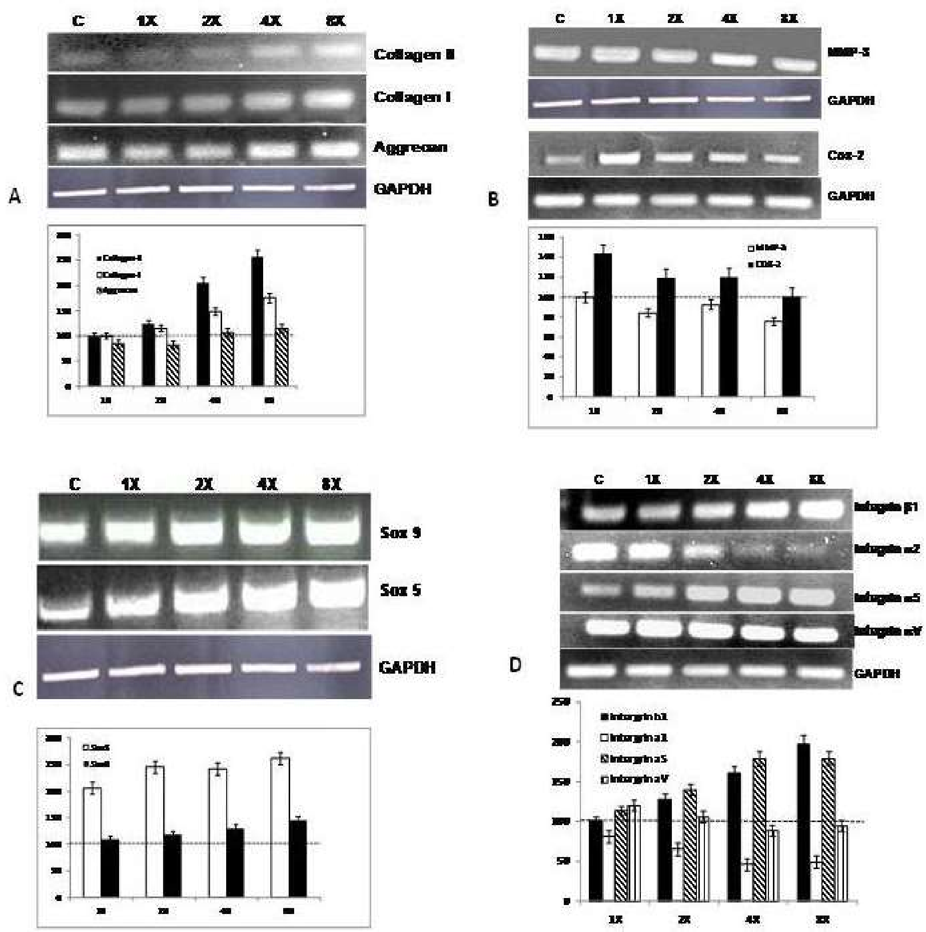

Figure 1. Analysis of the molecular responses of chondrocytes cultured in chitosan scaffolds to US stimulation. A 5.0 MHz US signal was applied 1-times, 2-times, 4-times, and 8-times per day for 51 seconds per application and maintained in culture for 10-days. The mRNA levels of indicated genes were measured by RT-PCR using specific primers as described by Hasanova et al. (2010). GAPDH gene was used as a loading control.

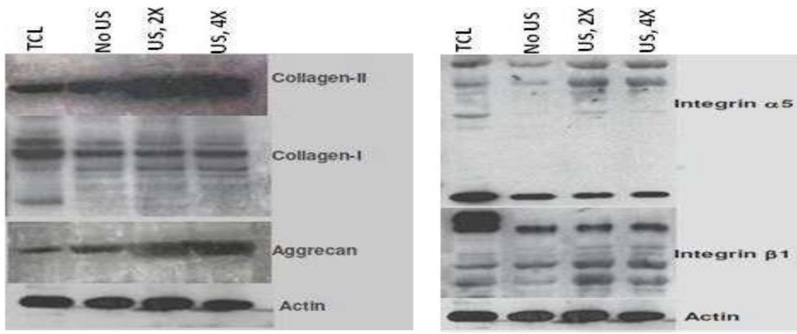

Figure 2. Protein expression analyses. The membranes were incubated with primary antibodies (1:1000 dilution) against type I and II collagen, aggrecan and integrins (β1 and α5). Actin (gamma) protein was used as the loading control. Protein bands were visualized using enhanced chemiluminescence (ECL) plus a detection system (Amersham Pharmacia Biotech, USA). TCL: Total cell lysate from bovine chondrocytes, No US: cells from non-stimulated controls, 2X: US stimulation was applied 2-time/day and 4X: US stimulation was applied 4-times/day.

conjunction with the related Sox-5 and Sox-6 genes (Bi et al., 1999). SOX factors (L-Sox-5, Sox-6 and Sox-9) are thought to be master regulatory genes that are essential for the expression of the chondrocyte phenotype. Thus, the gene expression of Sox-9 was assessed (Figure 1C) stimulated with US when compared to those without US stimulation (Figure 1B). Samples with cells stimulated with US for 8-times per day had 1.4- fold higher mRNA levels of Sox-9 when compared to control. Whereas, 1.9-fold to 2.5-fold higher levels of Sox-5 mRNA were noted in samples. The impact of US stimulation on the mRNA expression of matrix metalloproteinase-3 (MMP-3) and cyclooxygenase-2 (COX-2) was examined by RT-PCR at day 10 of the culture as a function of the frequency of US stimulus application (Figure 1B). MMP-3 is known to play a role in degradation of the ECM structure of cartilage, and our results indicate that gene expression of MMP-3 was not up-regulated by US stimulation. Similarly, US stimulation applied 2×, 4×, and 8× per day, did not affect gene expression of COX-2. Compared to the control, US application of 1× per day had a 1.3-fold higher level of COX-2 mRNA expression. These observations suggest that the conditions of US stimulation employed in our study did not significantly up-regulate the matrix degrading enzymes. Integrin signaling occurs in both directions: the binding activity is regulated from the inside of the cell (inside-out) resulting in the modulation of integrin expression, while the binding of the ECM elicits a cell response through the activation of cytoplasmic kinases (outside-in). Although several previous reports have addressed the role of integrins in mechanotransduction, there is a paucity of information detailing the effect of US on the expression of integrins by chondrocytes, both at the gene and the protein level. In this study, the modulation of inside-out integrin gene expression in response to US stimulation was evaluated. The mRNA expression levels of integrins β1, α2, α5, and αV in both non-stimulated control and US stimulated chondrocytes are shown in Figure 1D; and US stimulation was shown to impact inside-out integrin gene expression in a manner dependent on the frequency of application. The level of αV integrin gene expression was observed to be unaffected by US stimulation. Interestingly, when compared to the control, applications of US stimulation down-regulated the gene expression of integrin α2 in a frequency of application-dependent manner. For example, when compared to the control, 54%, and 51% decreases in the mRNA levels of integrin α2 were observed at US stimulation applied 4× and 8× per day, respectively. Integrins α5 and β1 were both up-regulated upon US stimulation in a frequency of application-dependent manner. Compared to the control, a 1.6-fold and 2.0-fold higher level of integrin β1 mRNA expression was observed US application at 4× and 8× per day, respectively. Similarly, when compared to the control, a 1.8-fold and 1.9-fold higher level of integrin α5 mRNA expression was observed when US was applied 4×, and 8× per day, respectively. Expression of chondrocytic proteins (collagen-II, collagen-I and aggrecan) and select integrins (α5 and β1) were analyzed by SDS-PAGE electrophoresis under reduced conditions, followed by western blots and shown in Figure 2. Total cell lysates obtained from plated bovine chondrocytes (passage-3) was used as a positive control. At the biosynthetic level, expression of chondrocytic proteins and integrins assayed increased upon US stimulation (Figure 2) and was consistent with mRNA expression analysis indicating that the increase in mRNA levels upon US stimulation correlated with their protein levels.

DISCUSSION

In this study, the US stimulation of chondrocytes in scaffolds was observed to up-regulate the gene expression of type-II collagen (CII) and GAG, chondrocytic markers. Interestingly, the gene expression analysis of chondrocytes stimulated with the US regimen (5.0 MHz, 51 sec^{-1} per application) indicated a co-coordinative up-regulation of Sox-5 and Sox-9 in response to US stimulation and generally mirrored the response of collagen type II transcript up-regulation to changes in US stimulation. Similar to observations reported elsewhere (Lee et al., 2006), these data demonstrate that in US stimulated chondrocytes, perhaps, up-regulation of collagen type II, the target of Sox-9, is due to enhancement of transcriptional levels of Sox-5 and Sox-9. In the same conditions, however, the mRNA expression level of MMP-3, a key matrix degrading enzyme, was down-regulated. Our data suggests that the US stimulation regimen employed induced the expression of chondrogenic markers, such as type-II collagen, aggrecan and Sox-9, indicating that US stimulation by itself could enhance chondrogenic differentiation in 3D culture. The response of chondrocytes to mechanical stimulation depends, in part, on the nature of the mechanical stimulus and a variety of different mechanotransduction pathways, with participation of different signaling molecules in the recognition and cellular responses to mechanical stimuli are likely to exist (Lee et al., 2000). In this paper, the analysis was confined to the hypothesis that integrins, cell-surface heterodimeric adhesion receptors that regulate cell viability in response to cues from extracellular matrix (ECM), are promising candidates for sensing ECM-derived mechanical stimuli and converting them into biochemical signals. While indirect mechanical stimulation in the form of an acoustic signal was observed to up-regulate the expression of integrins $\alpha5$ and $\beta1$ and down-regulate the expression of $\alpha2$ at the mRNA level, the connection between this response and other intracellular processes, as well as other pathways by which chondrocytes sense and react to mechanical stimuli, still needs to be defined. The up-regulation of integrins $\alpha5$ and $\alpha1$ was observed to correlate with the observed morphology on cell by SEM that has been reported elsewhere; cells on scaffolds under US stimulation were spread out, whereas cells on scaffolds without US stimulation had a spherical shape (Noriega et al., 2007). These collective findings lend credence to the idea that a variety of integrins are probably involved in the regulation of chondrocytic function in response to US stimulation. In conclusion, US stimulation at 5.0 MHz (0.14 mW cm^{-2}) for 51 sec per application was shown to modulate the proliferative capacity, biosynthetic activity, and integrin expression of human articular chondrocytes maintained in 3D matrices.

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Biological and Economic Peculiarities of Newly Developed Wheat Varieties

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Some aspects of recent achievements in wheat breeding in Azerbaijan gained at the Azerbaijan Research Institute of Crop Husbandsry (RICH) are given in the article. Long-term expedient and scientifically grounded breeding activities carried out by us have resulted in release of bread wheat varieties Azamatli-95, Nurlu-99, Gobustan, Ruzi-84 and promising varieties Gyrmyzy gul-1, Tale-38, Pirshahin-1 and Gunashli, which proved themselves in real field conditions that surves as a basis to increase grain crop production in the country. The main features of these wheat varieties include parameters like optimum assimilation surface area of leaves and non-leaf organs, providing sufficient attracting force to ears, resistance to abiotic and biotic stress factors, high productivity, short stalk and even stand of plants in cropped area.

Keywords: *wheat genotypes, phenological stages, architectonics, photosynthetic features, grain yield, quality*

INTRODUCTION

Bread is the main staple food source in Azerbaijan as in the other countries of the world. Many kinds of products made from wheat take main place in providing nutritional requirements to human population more than several thousands years. Thus, since XXI century due to high rate of the world population growth, urbanization processes and also global climate change wheat being strategic plant has occupied a main place in ensuring food security not only in Azerbaijan, but also all around the world.

Appropriately, abovementioned factors have led to twofold increase in demand for cereals and other agricultural products in the last 30 years. According to the last estimations of USDA wheat world production is predicted to be totally 656 million tons in 2009/10 that is 8% higher than that of the previous year.

At that point one of the priority trends in Azerbaijan agriculture is cultivation of grain crops among which wheat plays a leading role. It is cultivated in the area of more than 600 thousand ha in different regions of the Republic every year. Diverse soil-climatic condition of Azerbaijan has led to development of rich vegetation that allows considering Azerbaijan as a one of the possible centers of wheat origin (Vavilov, 1967).

At the beginning of research activities carried out in Azerbaijan wheat breeding was mainly based on the study of local populations and selection of valuable genotypes with useful agronomic traits that led to release of a number of durum and bread wheat varieties differing significantly from original ones. Intensive activities on wheat breeding have started at late 70s and early 80s of the last century. Breeding program embracing complex investigations which involved breeders, physiologists, biochemists, phytopathologists and other appropriate specialists has been adopted. As a result of long-term and efficient research activities "ideal" wheat type was elaborated, which determines optimum parameters of plant morpho-physiological characters and architectonics (Aliyev, 1974, 1983; Aliyev and Kazibekova, 1979, 1988).

Taking the soil-climatic diversity of Republic into account new breeding program with following priority trends have been adopted in Research Institute of Crop Husbandry to solve the problems caused by global climate change:

- Acceleration of the breeding process through benefiting the world experience on plant breeding;
- Plant variety testing with the purpose of identifying the most flexible varieties with low response to diverse soil-climatic environment;
- Extension of the fundamental researches in order to develop physiological and biochemical basis against different stress factors, resistance and productivity and use of them in evaluation of breeding material.

Presently basic and applied research activities have been going on at Research Institute of Crop

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Husbandry towards successful implementation of the plant breeding program.

1996 year was crucial in execution of this program after official establishment of scientific cooperation with International Scientific Centers as CIMMYT (International Maize and Wheat Improvement) and ICARDA (International Center for Agriculture Research in Dry Areas).

Every year more than thousands of durum and bread wheat accessions are introduced from different research centers that influenced positively on the establishment of unique wheat gene pool. The main value of this gene pool is determined by its specifics and opportunities for investigations of genetics of photosynthesis, study of heritability and heredity of the photosynthetic traits, for distinguishing the donor characteristics of genotypes, and, most importantly, for the complex implementation of the breeding wheat varieties close to "ideal" type.

A conception has been elaborated as a result of studies on diversity of wheat with different values of photosynthetic traits, productivity and morpho-physiological characteristics of plants that finally allowed to determine high productivity of "ideal" type wheat variety. Hence, breeding activities conducted in Azerbaijan enabled to develop a number of wheat varieties with potential yield of 7.5 – 8.0 t ha^{-1} in irrigated agriculture. Apart from old wheat varieties Barakatli-95, Giymatli-2/17, which have been grown for many years new, high yielding wheat varieties including Azamatli-95, Nurlu-99, Gobustan, Ruzi-84, Tale-38 have been released and are widely grown in the country and promising ones like Gyrmyzy gul-1, Pirshahin-1, Gunashli and etc. have been submitted to the State Commission for Protection and Testing of the Breeding Achievements.

In this aspect the study and evaluation of the biological and agronomic traits of the different newly developed bread wheat varieties contrasting in architectonics have a great theoretical and practical importance in breeding.

MATERIALS AND METHODS

Wheat varieties contrasting in architectonics were developed through selection of ten thousands of materials from different gene pools including from local and that of introduced from CIMMYT, ICARDA and other International Breeding Centers. Plants were grown in diverse soil-climatic environmen of the Republic at the optimum regime of the mineral nutrition for the given area (N_{90-120}; P_{90-100}; K_{60} e.m.). To maintain soil moisture content in irrigated agriculture at the level of 70-75% during vegetation period 2-3 supplemental irrigation has been provided depending on the year. Comparative biological characterization of the earlier and newly developed wheat varieties grown in irrigated agriculture was conducted at Absheron Experimental Station of RICH. Field testing was carried out in Regional Experimental Stations of RICH located in diverse soil-climatic environments including in Tartar (irrigated agriculture), Gobustan (dry rainfed) and Jalilabad (humid rainfed).

Phenological observations were carried out on plant growth and performance, assimilation surface area, intensity of photosynthesis (P_I) and dry biomass accumulation, photosynthetic potential and specific leaf density during ontogenesis.

Phenological observations of wheat varieties were made according to Kuperman (1997), assimilation surface area was measured using automatic device "AAC-400" (Japan), and CO_2 assimilation intensity was measured by infrared gas analyzer "URAS-2T" (Germany) (Voznesenskiy et al., 1965; Sestak et al., 1971).

Photosynthetic potential (P_p) was calculated using standard formula (Grichenko and Dolqodvorov, 1986), specific leaf density (SLD) was determined using cutting method by the ratio of cut leaves weight to their area (Rasulov and Asrorov, 1982). Dry biomass of samples was calculated by absolute dry mass, and grain yield - by planting area unit. Grain protein content was determined by standard method (Ermakov et al., 1972).

RESULTS AND DISCUSSION

Wheat varieties contrasting in architectonics have sharply differed by the length of phenology phases during vegetation (Table 1). This is specially expressed in period of node formation during tubing stage, at the beginning of heading, in the sum of days from heading till complete maturity of grain and vegetation period. The intensity of node formation during tubing is not identical for all studied wheat varieties. During intercalar growth most durable node formation was observed in newly developed bread wheat varieties like Nurlu-99, Gobustan, Azamatli-95, Ruzi-84 with high architectonics that provide favorable conditions for growth and development. It varied between 41-54 days.

Similar results were obtained in earlier developed intensive type durum wheat variety Barakatli-95. Newly developed varieties, which are widely grown in the country affect positively on the duration of the whole period of heading/complete grain maturity stages. In irrigation conditions it lasts around 60-64 days that is favourable to duration of grain formation and grain filling, so these varieties ears on average 10-

20 days earlier than others. Such difference in development and growth of earlier and newly developed wheat varieties with various bio-morphological features has reflected on their vegetation. Vegetation period of studied wheat varieties has fluctuated between 218-233 days. For newly developed wheat varieties with good architectonics this period has been 218-227 days, which 5-13 days shorter than that of extensive traditional varieties Gyrmyzy bugda and Shark, which dominated in the country by cropped area till 1970. This is considered the main reason of low yielding (0.7-0.9 tha^{-1}) at that time.

Comparative description of principle maximum values of leaf photosynthetic activity of earlier and newly developed wheat varieties is given in Table 2. Newly developed varieties distinguished by optimum growth that favoured better photosynthetic activity during vegetation.

Leaves of newly developed short-stemmed wheat varieties with high architectonics were found mostly, narrow, short and vertical oriented. Maximal values of leaf photosynthetic traits of studied wheat varieties were observedf at one of yield responsive period of ontogenesis, intermediate between heading and flowering stages.

Optimum value and vertical orientation of leaves favour more durable activity and effective use of solar energy, and especially high photosynthetic activity of different horizontal layers of sowings. Compared wheat varieties Gyrmyzy bugda and Giymatli-2/17 developed wider than optimum leaf surface area under irrigation. Maximal value of leaf area reached up to 79.5 and 78.5 th.m^2 ha^{-1}, respectively. Positive typical characteristic of photosynthetic activity of newly developed varieties affected photosynthetic potential, photosynthetic intensity and specific leaf density (Aliyev and Kazibekova, 1979, 1988, 2002).

The maximal value of leaf photosynthetic potential as one of the crucial factors determining yield capacity at the heading and flowering stages were 550-686 th.m^2 day ha^{-1} for newly developed wheat varieties. As shown in the Table 2, the maximum value of photosynthetic potential of wheat variety Giymatli-2/17 (1038 th.m^2 day ha^{-1}) and extensive type Gyrmyzy bugda (83.8 th.m^2day ha^{-1}) was on average 30-40%, which is higher in comparison with new varieties.

Maximum value of flag leaf photosynthesis intensity is observed mainly at the heading/flowering stages and varied within 25.0-34.0 mg CO_2 dm^{-2} h^{-1} in newly developed wheat varieties. The same is observed in following stages of development. The difference between newly developed and extensive type varieties was observed 20-35% in other stages too (Table 2).

Similar results for value of specific leaf density (SLD) were obtained in newly developed wheat varieties. Being an essential photosynthetic trait in ontogenesis, SLD varied in a wide range (0.576-0.676 g dm^{-2}) that affected effective use of assimilates for leaf growth during formation of assimilation surface area. The maximum value of SLD was obtained in newly developed and registered bread

Table 1. Development and growth variation features of earlier and newly developed wheat varieties cultivated in irrigation conditions of Absheron

No	Variety	*Period of node formation	Date		Sum of days from heading to complete grain maturity	Vegetation period
			Heading	Complete grain maturity		
1	Gyrmyzy bugda	17.03 -23.04 38	09.05-14.05	24.06-27.06	45-47	230-233
2	Barakatli -95	08.03 – 16.04 40	25.04– 27.04	18.06-21.06	55-56	224-227
3	Giymatli-2/17	28.02 – 09.04 41	17.04– 28.04	15.06–19.06	61-62	221-225
4	Nurlu-99	08.02 – 02.04 54	19.04– 15.04	12.06–14.06	57-60	218-220
5	Azamatli-95	28.02 – 11.04 43	20.04–23.04	19.06–21.06	60-61	225-227
6	Gobustan	18.02 – 08.04 50	14.04–17.04	15.06–19.06	63-64	221-225
7	Ruzi-84	28.02 – 09.04 41	16.04-20.04	18.06–21.06	63-64	224-227
8	Gyrmyzy gul-1	21.03 – 22.04 33	30.04– 04.05	22.06–24.06	52-54	228-230
9	Tale-38	18.03 – 23.04 37	01.05– 07.05	21.06–24.06	49-52	227-230

*In numerator periods are given, in denominator sum of days from I to V node formation is given

Table 2. Comparative characteristics of main parameters of photosynthetic activities of leaves of earlier and newly developed wheat varieties cultivated in irrigation conditions of Absheron

No	Variety	Leaf area, th.m^2 ha^{-1}	P_P, th.m^2 day ha^{-1}	(SLD), g dm^{-2}	P_I, mg CO_2 dm^{-2} h^{-1}	Leaf dry biomass, t ha^{-1}	C_{econ}, %
1	Gyrmyzy bugda	79.5	838	0.452	20.5	4.62	16.1
2	Barakatli-95	62.7	605	0.638	28.5	4.01	35.9
3	Giymatli-2/17	78.7	1038	0.513	25.8	4.07	31.1
4	Nurlu-99	48.8	564	0.577	25.4	3.11	38.2
5	Azamatli-95	50.6	585	0.676	32.2	3.53	38.2
6	Gobustan	65.4	631	0.558	27.3	4.18	37.7
7	Ruzi-84	59.4	573	0.576	25.2	3.79	32.0
8	Gyrmyzy gul-1	45.3	550	0.634	27.0	2.85	40.1
9	Tale-38	69.9	686	0.596	33.3	4.35	37.0

wheat variety Azamatli-95 (0.676 g dm^{-2}) and promising variety Gyrmyzy gul-1 (0.634 g dm^{-2}). In extensive durum wheat variety Gyrmyzy bugda the value of SLD (0.452 g dm^{-2}) was on average 20-30% lower than that in newly developed varieties (Table 2).

In general, the values of photosynthetic traits of newly developed wheat varieties with good architectonics were high at the grain filling period due to durable and fruitful functioning of leaf assimilating area. The difference of photosynthesis activity was reflected in the formation of dry biomass, potential yield and its components.

The studied wheat varieties differed on dry biomass accumulation. In modern wheat varieties during vegetation accumulation of dry biomass in the leaves is relatively longer, because vital activity of leaves from higher layers is more durable in comparison with earlier developed varieties. The highest value of leaf dry biomass was, mainly, obtained in earlier developed varieties, particularly in extensive variety Gyrmyzy bugda (4.62 t ha^{-1}) (Table 2). In comparison with other varieties this one produced 20% more dry biomass. While the increase of dry biomass in long-stemmed variety Gyrmyzy bugda mainly occur by vegetative parts, that causes to decrease in contribution of economic yield components to total biomass.

The grain yield of different wheat varieties significantly varies dependent on biological features, soil-climatic conditions and vegetation year (Figure 1). According to long-term investigation data average grain yield obtained under irrigation at the Absheron Experimental Station of RICH has constitued 3.34-6.93 t ha^{-1}. Newly developed varieties produced high grain yield, which varied within 5.88-6.93 t ha^{-1} due to optimum value and vertical orientation of leaves, stable functioning of assimilating organs, especially in reproductive stage of vegetation. In this case, in extensive long-stemmed durum wheat variety Gyrmyzy bugda (var. *hordeiforme* Host.) with poor architectonics, large assimilating surface area of leaves and other non-leaf organs negatively affected not only on photosynthetic activity of different horizontal layers of sowings, but also on grain yield formation too. The grain yield of Gyrmyzy bugda has made up 3.0 t ha^{-1}. The difference in yield value among extensive and newly developed wheat varieties was two fold more.

Similar results on grain yield production were obtained in newly developed wheat varieties, cultivated in diverse soil-climatic conditions of the Republic. With the purpose of determining the yield potential, studied wheat varieties were exposed to field tests at the Experimental Stations of RICH which characterizes soil-climatic conditions of Azerbaijan Republic and seed multiplication of these varieties was arranged at state and private seed-farms. It was very important to determine potentials of new high-yielding and high-quality varieties suitable to local ecological conditions of each region. Hence, it was important to develop theoretical and methodical bases to develop new intensive type of high-yielding crop varieties. As shown in Figure 2, new bread wheat varieties have produced high yield at each ecological condition.

New wheat varieties with favourable architectonics like Azamatli-95, Tale-38, Gyrmyzy gul-1, Pirshahin-1 have produced high grain yield (7 t ha^{-1} and more) in irrigation conditions. These varieties were also adopted by small scale farmers.

Growing ecological disbalance and environmental stress factors require developing more suitable wheat that can be grown in all environments available in the country. Drought is becoming main abiotic stress factor in the Republic limiting the yield of agricultural crops. The grain yield of new drought tolerant bread wheat varieties like Ruzi-84, Gobustan, and Gunashli has constituted 4.92-5.50 t ha^{-1} in dry rainfed agricultural environment (Figure 2).

Similar results were observed in the ratio of grain yield/straw. As such, in new wheat varieties

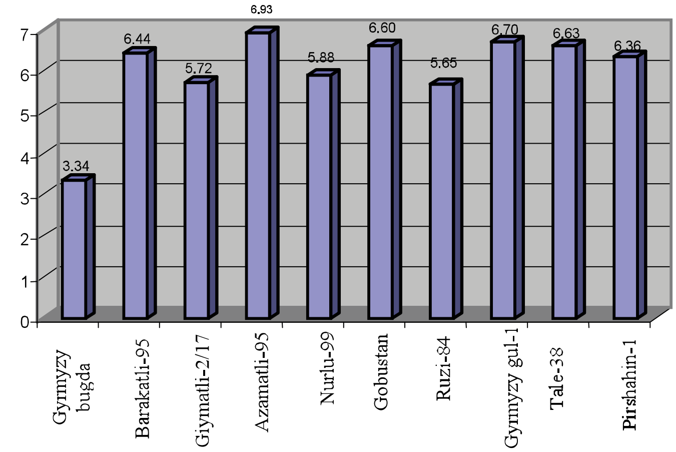

Figure 1. Comparative characteristics of grain yield of different old and newly developed wheat varieties in irrigation conditions of Absheron (t ha^{-1}) (on average).

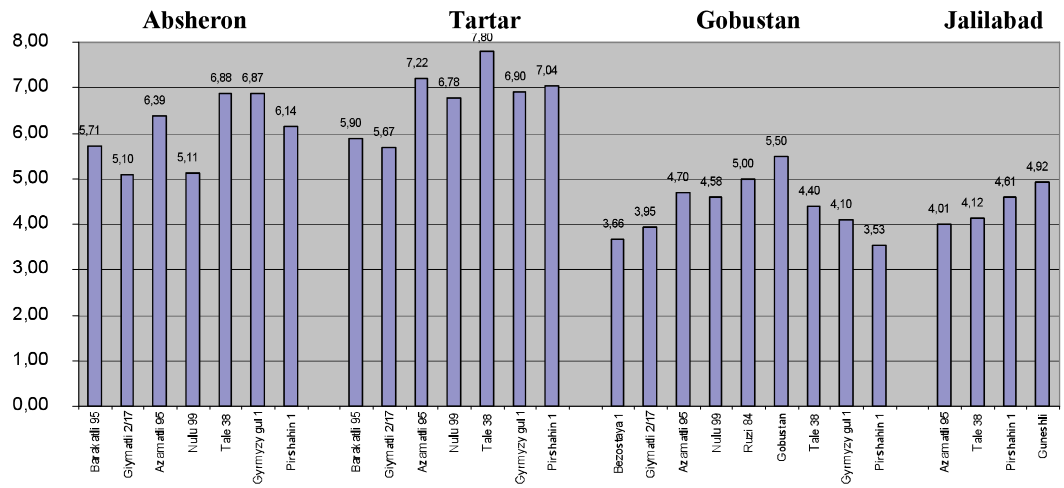

Figure 2. Comparative characteristics of grain yield of old and newly developed wheat varieties grown in multiplication nursery in diverse soil-climatic regions of Azerbaijan Republic (t ha^{-1}) (on average).

grain yield contribution to biological productivity varied from 33.0 to 40.0% that is on average 21.0% higher than that of long-stemmed extensive type wheat variety Gyrmyzy bugda. In Gyrmyzy bugda dry biomass has increased mainly on account of vegetative organs that causes decrease in contribution of important yield components to total biomass. Thus, maximum value of economic efficiency coefficient (C_{econ}), which is important resource for increase in productivity increase, was observed in newly registered and promising bread wheat varieties Nurlu-99, Azamatli-95, Tale-38, Gobustan, Gyrmyzy gul-1 that varies between 37.0-40.1% (Figure 3).

Comparative characterization of spike structural elements dependent on cultivation area is also given in Table 3. Bread wheat varieties Giymatli-2/17, Azamatli-95 and Nurlu-99 were exposed to multilocation variety testing in different soil-climate environments of Azerbaijan, i.e. in Absheron, Tartar and Nakhchivan. It was found out that bread wheat variety Azamatli-95 performed almost all its potentials in Nakhchivan, particularly in number and weight of grain per spike. Here this variety had record number (75.4) and weight (3.04 g) of grain per spike (Table 3). 1000 kernel weight as a one of the yield elements was comparatively studied and evaluated in new wheat varieties. The intensive durum wheat variety Barakatli-95 in comparison with bread wheat variety has distinguished by 1000 kernel weight, which was 50.3 g that is 10-15% more. Maximum value of 1000 kernel weight was observed in new bread wheat varieties Tale-38, Azamatli-95 and Ruzi-84, varying between 46.4-47.4 g (Table 4).

Determination of protein content plays an important role when characterizing wheat varieties (Pavlov, 1982; Aliyev and Mahmudov, 1992). Wheat varieties

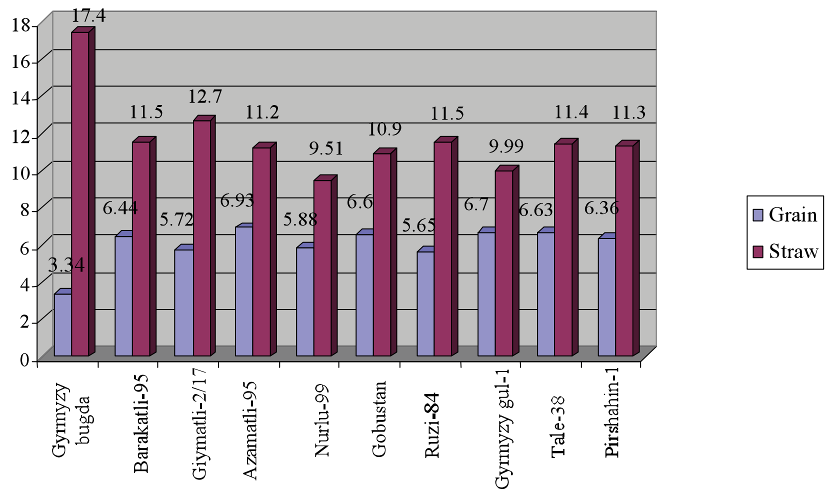

Figure 3. Comparative characteristic of grain yield correlation to straw in old and newly developed wheat varieties cultivated in irrigation conditions of Absheron (t ha^{-1}) (on average).

studied in Absheron under irrigated agriculture distinguished themselves by protein content. As such, final direction of plant nitrogen metabolism is more clearly determined by grain yield structure and quality (Table 4). It consists of traits which are controlled by different particular genes (Bhatia, 1975; Konarev, 1980; Krasnaya, 1983). Protein quality has connection with other yield elements. Hence, we comparatively studied protein productivity of varieties with link between grain yield and protein content.

It was observed that in durum and bread wheat varieties protein content of grain, protein production per hectare varied in wide range. Durum wheat variety Gyrmyzy bugda (14.9%) and bread wheat variety Azamatli-95 (14.0%) had high protein content. Discrepancy of grain yield value has influenced on protein content per sowing area unit. Though extensive variety Gyrmyzy bugda had high protein content, it conceded in protein production more than twice than other wheat varieties with high architectonics. New wheat varieties Azamatli-95, Gyrmyzy gul, Gobustan and Tale-38 had high protein productivity, which varied between 825-970 kg ha^{-1} (Table 4).

Thus, long-term breeding activities has resulted in development of new crop varieties which approved themselves in real field condition in diverse soil-climatic environments of Azerbaijan and surved as basis for increasing crop production in the country. Brief characteristics of newly developed and registered wheat varieties are given below.

Azamatli-95 belongs to var. *graecum Körn.* Short-stemmed (90-95 cm), relatively early mature, tolerant to drought, resistant to yellow rust, vegetative period is 225-227 days, in irrigated agricultures yield potential is 7.5-8.5 t ha^{-1}, 1000 kernel weight is 45-47 g, protein content is 14.0-15.0%, gluten content is 30-32%.Variety is recommended to be grown, mainly in irrigated, rainfed agriculture and foothill zones. Variety has been are registered by State Commission for Testing and Protection of Breeding Achievements in 2004.

Nurlu-99 belongs to var. *graecum Körn.* Short-stemmed (80-85 cm), early mature, moderate resistant to fungal diseases, vegetation period is 215-220 days, in irrigation conditions potential yield is 7.5-8.0 t ha^{-1}, 1000 kernel weight is 38-40 g, protein content is 13.5-14.0%, gluten content is 30-35%. Variety is recommended to be grown mainly, in irrigated regions of Republic. Variety has been are registered by State Commission for Testing and Protection of Breeding Achievements in 2004.

Gobustan belongs to var. *graecum Körn.* Short-stemmed (80-90 cm), early mature, tolerant to drought, resistant to rust diseases, vegetation period is 220-225 days, in irrigation conditions potential yield is 7.0-8.0 t ha^{-1} and 4.5-5.5 t ha^{-1} in rainfed conditions, 1000 kernel weight is 40-44 g, protein content is 12.5-13.5%, gluten content is 28-30%. Variety is recommended to be grown, mainly, in rainfed, semiarid and irrigated regions of Republic. Variety has been are registered by State Commission for Testing and Protection of Breeding Achievements in 2006.

Ruzi-84 belongs to var. *graecum Körn.* Moderate-stemmed (97-103 cm), relatively early mature, tolerant to drought, winter hardy and resistant to fungal diseases, vegetation period is 224-227 days, in rainfed conditions potential yield is 4.5-5.0 t ha^{-1}, 1000 kernel weight is 38-42 g, protein content is 12.0-13.0%, gluten content is 26-28%. Variety is recommended to be grown basically in rain-fed, semiarid and foothill regions of the Republic. Variety has been registered by State Commission for Testing and Protection of Breeding Achievements in 2006.

Table 3. Comparative characteristic of ear structural elements of old and newly developed and registered bread wheat varieties dependent on cultivation area

No	Variety	Location	Ear length, cm	Ear width, cm	Number of spikelets per ear	Ear weight, g	Number of grains per ear	Grain weight per ear, g
1	Giymatli-2/17	Absheron	9.50	1.70	21.4	4.80	55.2	2.90
		Tartar	9.10	1.55	22.2	3.90	41.6	1.80
		Nakhchivan	7.78	1.40	19.8	3.70	58.8	2.80
2	Azamatli-95	Absheron	12.3	1.20	17.8	2.90	41.4	1.80
		Tartar	12.0	1.25	21.5	3.15	51.8	1.90
		Nakhchivan	13.0	1.40	20.2	4.80	75.4	3.04
3	Nurlu-99	Absheron	9.40	1.40	18.2	2.60	44.8	1.70
		Tartar	9.35	1.45	20.9	2.91	54.3	2.01
		Nakhchivan	8.70	1.20	16.6	2.70	46.6	1.80

Table 4. Comparative characteristics of protein productivity at old and newly developed wheat varieties cultivated in irrigation conditions of Absheron (on average)

No	Variety	Grain yield, $t \cdot ha^{-1}$	Protein content, %	Protein productivity, kg ha^{-1}	1000 kernel weight, g
1	Gyrmyzy bugda	3.34	14.9	498	43.7
2	Barakatli-95	6.44	13.5	869	50.3
3	Giymatli-2/17	5.72	13.7	784	47.9
4	Azamatli-95	6.93	14.0	970	46.8
5	Nurlu-99	5.88	13.7	806	39.2
6	Gobustan	6.60	12.5	825	44.1
7	Ruzi-84	5.65	13.2	746	46.4
8	Gyrmyzy gul-1	6.70	13.5	905	41.5
9	Tale-38	6.63	13.1	869	47.4
10	Pirshahin-1	6.36	13.2	839	46.7

Gyrmyzy gul-1 biologically is winter type variety, has high architectonics, and belongs to var. *erytrospermum Körn*. Short-stemmed (84-88 cm), relatively late mature, vegetation period is 228-230 days, in irrigation conditions potential yield is 7.0-8.0 t ha^{-1}, 1000 kernel weight is 41-46 g, protein content is 13.5-14.0%, gluten content is 26.8-28.2%. It performed moderate susceptibility to leaf rust in different years. It has been submitted to State Commission for Testing and Protection of Breeding Achievements in 2005.

Tale-38 biologically is winter type variety, has high architectonics, and belongs to var. *graecum Körn*. Short-stemmed (92-97 cm), relatively late mature, vegetation period is 227-230 days, in irrigation conditions potential yield is 7.5-8.5 t ha^{-1}, 1000 kernel weight is 44-49 g, protein content is 13.0-14.0%, gluten content is 26.2-27.2%. Resistant to fungal diseases. Variety is recommended to be grown in irrigated and humid rainfed agriculture including mountain and foothill regions of Republic. Variety has been submitted to State Commission for Testing and Protection of Breeding Achievements in 2005.

Pirshahin-1 belongs to var. *graecum Körn*. Short-stemmed (94-98 cm), relatively early mature, winter hardy, tolerant to lodging and resistant to diseases, vegetation period is 222-226 days, in irrigation conditions potential yield is 7.5-8.5 t ha^{-1}, 1000 kernel weight is 29.8-31.2 g, protein content is 13-14%, gluten content is 29.8-31.2%. Variety is recommended to be grown in irrigated and humid rainfed agricultural system. Variety has been submitted to State Commission for Testing and Protection of Breeding Achievements in 2006.

Gunashli belongs to var. *erytrospermum Körn*. Short-stemmed (81-88 cm), relatively early mature, tolerant to a drought and lodging, winter hardy, and resistant to diseases, vegetation period is 223-226 days, in rainfed agricultural system potential yield is 4.5-5.5 t ha^{-1}, 1000 kernel weight is 46-50 g, protein content is 14.5-15.0%, gluten content is 28.0-29.8%. Variety is recommended to be grown in humid rainfed agricultural system. Variety has been submitted to State Commission for Testing and Protection of Breeding Achievements in 2006.

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