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RESEARCH PAPERS

QTL Identification and Mapping in Soft Spring Wheat (*Triticum aestivum* L.) under Controlled Agroecological and Biological Testing Area Conditions with and without Nitrogen Fertilizer

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Abstract—Quantitative trait loci (QTL) of agriculturally valuable traits of soft spring wheat (*Triticum aestivum* L.) were mapped in two simultaneous and independent experiments that were carried out in different agronomical backgrounds with respect to nitrogen availability (i.e., with and without introduction of a mineral nitrogen fertilizer) in order to reveal the effects of physiological and genetic interaction between the genotype and the environment. In total, 94 QTLs, which determine 31 physiologically and agriculturally important traits, have been identified. The connection between the loci identified and polymorphism by certain traits has been proven. The connection between the trait expression and introduction of the fertilizer has been confirmed by both correlation analysis and the single-factor analysis of variance. The analyses of QTL and correlation, as well as the single-factor analysis of variance, showed that 15 of 31 traits varied confidently. This shows that the expression of these traits depends on the presence of nitrogen nutrition. The data obtained are important for further study of physiological and genetic regulatory mechanisms of expression of the traits that were evaluated in the system of interaction between the genotype and the environment as well as for the marker-assisted selection of wheat.

Keywords: Triticum aestivum, nitrogen fertilizers, physiologically and agronomically valuable traits, QTL mapping, controlled conditions of agroecological and biological testing area, genotype-environment interaction

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most wide spread agriculturally important plant species all over the world. It is the most important source of proteins and carbohydrates for people. Nitrogen fertilizers are crucially important to increase wheat productivity and crop yields as well as to increase the level of proteins. According to the prognoses of the International Fertilizer Industry Association (IFA) and the Food and Agriculture Organization of the United Nations (FAO), the worldwide demand for mineral fertilizers will reach 200.5 million t by 2018 [1]. It is expected that the demand for nitrogen fertilizers will grow by 1.4–1.5% annually, and it will exceed 1 million t per year in certain regions [2]. However, it is also expected to vary, depending on climate and changes in market prices. Therefore, one of the main goals of modern selection is to increase the efficacy of nitrogen fertilizers in order to achieve high productivity of wheat. The efficacy of assimilation of nitrogen fertilizers is a complex trait, which is controlled by numerous genes and depends on the "genotype-environment" interaction. Estimation of the effect of nitrogen fertilizers on different genotypes is one of the most promising approaches to achieve high productivity of plants [3]. To improve qualities of nitrogen fertilizers, in addition to the conditions and quantities of fertilizers introduced, it is important to bear in mind not only the cultural genotype but also environmental effects and interactions between all these three factors [4].

QTL-mapping provides the possibility of the genetic control over the quantitative traits and genes that regulate them. A number of QTLs have been identified at low and high doses of nitrogen nutrition under different conditions of cultivation. For example QTLs in wheat were found under both regulated [5–7] and field conditions [8–10]. The study, which was carried

Abbreviations: ITMI—International Triticeae Mapping Initiative; LOD—logarithm of odds; QTL—Quantitative Trait Loci; RILs—recombinant inbred lines; sM—centi-Morgan unit; VIIs—vegetation irradiation installations.

out by an international research team [6], was aimed at the QTL-mapping of 21 traits connected with growth, productivity, and assimilation of nitrogen by leaves during the formation of crops in hexaploid wheat using a mapping population, which was obtained by the Chinese Spring and the SQ1 crossing (the breeding line that expresses a high level of abscisic acid). As a result of this study, the main QTLs that determine the activity of glutamine synthetase, the number of spikelets in the ear, the number of flowers, and the number of seeds and plant productivity were identified in the chromosomes 2A, 4A, and 6B. Chinese scientists [11] performed the QTL-mapping of productivity connected with nitrogen consumption in wheat. These traits were located in chromosomes 2D, 4B, 4D, 5A (2), 6A, and 7A and demonstrated considerable connection between the crop and seedling traits, the level of nitrogen and the efficacy of its consumption. A little bit earlier, French scientists [12] identified 54 motives located in almost all chromosomes and that affected yield and its components, plant height, earing period, and concentration of protein in a grain. It was proposed to use these motives in selection programs aimed at the improvement of the existing wheat breeds that are adapted to the modern nitrogen fertilizers [13]. They may also serve as a source for the positional cloning of genes, which are involved in the effective assimilation of nitrogen fertilizers.

The vegetative experiment, which was carried out under the field conditions, dealt with identification and chromosome mapping of the loci responsible for the expression of physiologically and agronomically important traits, the manifestation of which depends on the efficacy of consumption of mineral nitrogen introduced into soil [27]. It was shown that the activity of genetic determinants that determine the expression of physiological and agronomical traits depends on the dose of mineral nitrogen supplied (nitrate and ammonia forms of nitrogen). The experiment was carried out under field conditions in Leningrad region in 2009. This region is characterized by unstable climatic conditions that do not allow excluding the effect of changes in temperature, irregular precipitations, and variations in soil humidity on the localization of QTL-traits that are connected with morphological and physiological features, growth rate, and productivity of wheat [27]. However, all these studies do not allow us to perform the "precise" mapping of the chromosome loci that are involved in the regulation of nitrogen consumption under different environmental conditions. Use of an agroecological and biological testing area with strictly controlled growing conditions provides this possibility [14]. In contrast with the aforesaid papers [5–7], in which the regulated conditions were represented by either hydroponic culture of greenhouse, an agroecological and biological testing area is an installation that is fully isolated from sunlight and any other outer influences by the vegetative chambers [14]. An agroecological and biological testing area is equipped with the systems of accurate microclimatic control, the corresponding vegetation irradiation equipment of different types for all-year intensive growing of plants of different heights (the latter is especially important for agricultural plants), and the devices for the remote and contact diagnostics of physiological and morphological conditions of the vegetative plants. This allows us to conduct physiological and genetic experiments with a large number of plants under the strongly regulated conditions of an agroecological and biological testing area not only in individual vegetative chambers but also in the specially designed shelves filled with peat. This is the advantage of an agroecological and biological testing area in comparison with hydroponic culture and different types of greenhouses, including the so-called "phenomix-greenhouse" [14]. However, up till now, no study was carried out in which the genetic determinants that determine the quantitative trait expression responsible for the consumption and assimilation efficacy of nitrogen fertilizers would have been performed under the regulated conditions of an agroecological and biological testing area.

The present study was aimed at estimating of the number and precise location of QTLs that are involved in the physiological and genetic processes of regulation of complex agriculturally important traits of soft spring wheat (*Triticum aestivum* L.) and that are expressed under the regulated conditions of agroecological and biological testing system with and without nitrogen fertilizer.

MATERIALS AND METHODS

Plant material. The objects of the study were the recombinant inbred lines (RILs) of the mapping population of soft spring wheat (*Triticum aestivum* L.) of the International Triticeae Mapping Initiative (ITMI). The ITMI mapping population was obtained by pollination of soft spring wheat *Triticum aestivum* L. of the Opata 85 variety with pollen of the synthetic hexaploid W7984, the amphiploid obtained by crossing of *Aegilops tauschii* Coss., sample CIGM86.940 (*DD*) (paternal line) and tetraploid wheat *T. turgidum* var. *durum,* variety Altar 84 (*AABB*) (maternal line) as described in [14–16].

Growing conditions. Growth and assessment of the ITMI inbred lines were performed under the regulated conditions of the biological agroecosystem designed in the Agrophysical Research Institute [14]. 114 lines of the ITMI mapping population and their parental forms Opata 85 and the synthetic hexaploid W7984 was performed in the vegetative irradiation installations equipped with the DNaT-400 lamps, which provide adjustable irradiation. The installations were placed in the stationary building isolated from natural light, equipped with heating system and balanced system of ventilation. The main life sustenance parameters were kept constant throughout the cultivation

period. The temperature regime was sustained as follows: 25–26°C day/20–21°C night. The photoperiodic regime included the 16 h illumination daily. Both temperature and photoperiodic regimes of plant growing were chosen so as to create conditions that would have leveled the genetic differences between the samples studied that determine the reaction to this factors. The irradiation at the level of the top leaves was 50 ± 0.5 W/m^2 FAR. During the plant growth, the irradiation was corrected by changing the distance between the lamps and plants and was kept constant throughout the vegetation period. Plants were grown in 2 L vegetation vessels. Two plants were settled in each vessel so as to get 100 plants per 1 m^2. The experiment was repeated twice for each line and for each parental form.

The experiment was carried out on sod-podzol, slightly loam soil that contained 198 mg/kg mobile phosphorus (GOST P 54650-2011), 112 mg/kg mobile potassium (GOST P 546650-2011), 18.2 mg/kg nitrate (GOST 26951-86), and 34.6 mg/kg ammonia (GOST 26489-85). The additional supplementation of soil with macroelements was not performed because of increased level of mobile phosphorus and potassium. The experiment included two variations that differed from each other by the level of nitrogen nutrition. In the first variation (low level), nitrogen fertilizers were not introduced during the vegetation period, while two-fold supplementation with urea (GOST 2081-2010) was performed before sowing and in the stem elongation phase of ontogenesis in the second variation (high level). In two stages, 0.321 mg of urea per 1 kg soil were introduced during the additional fertilization. The humidity of soil was sustained at the optimal level (70–80% of total water-absorbing capacity at daily watering) throughout the vegetation period. Phenological observations were performed once in 2 days and daily within the main ontogenetic phases. At the end of the vegetative experiments, the main productivity parameters were assessed for each plant.

Analysis of traits. The analysis of traits was performed in accordance with the methods accustomed in the Vavilov All-Russia Institute of Plant Genetic Resources as described in [14–16]. Only those traits that demonstrated a sufficient level of expression were taken into consideration. In total, 31 traits expressed throughout the vegetation period were analyzed (Table 1).

Statistical analysis. The QTL-analysis was carried out with the computer program MAPMAKER/QTL. The mapping data published in the GrainGenes database (gopher:http://www.greengenes.cit.cornell.edu) were used to recalculate the distances on the map with the MAPMAKER/EXP 3.0 [18], because the MAPMAKER/QTL program performs calculations using the mathematical formula introduced by Haldane [17]. The obtained data of phenotypic analysis were integrated into the existing base map that was composed for the ITMI population [19]. Identification and localization in the linkage groups were performed with the QGENE program described in [14, 20], using only the markers that corresponded to the mapping function by D.D. Kosambi, which takes interferention into account [21].

Confidence of the connection between the loci identified and the polymorphism of certain traits was assessed on the basis of a threshold value of the ration of logarithm of odds likelihood [14, 22, 23]. Individual QTL-analysis was performed for each train in each experiment, and the trait variation degrees (R^2), which are explained by the QTL-data, were estimated. Confidence of each LOD was estimated by the permutation test (1000 repeats). Only those loci whose LOD values were ≥3.0 ($P < 0.001$), $2 < \text{LOD} < 3$ ($P < 0.01$), and $1.5 < \text{LOD} < 2$ ($P < 0.1$) were taken into account [14, 15].

To estimate the level of connection between each trait and introduction of a nitrogen fertilizer, the correlation coefficient r_{xy} was calculated. The ratio of r_{xy} to its error was used to estimate the confidence level (Student's t-test) [24]. To perform the complex assessment of the compared mean values of traits, which were estimated at different growth conditions, the analysis of variances was carried out, taking into account the variation indicators, particularly mean square deviation, their dispersion ratio F, and confidence of the data obtained [25]. The acceptable threshold of the statistic confidence was considered to be $P < 0.05$, because this level of confidence includes 5% probability of a mistake. Data that were confident at $P < 0.01$ were considered to be statistically reliable, and the data at $P < 0.001$ were considered as highly confident. All calculations were carried out with STATISTICA 6.0.

RESULTS

Our study revealed significant variations in agronomically important traits in the same genotypes of the ITMI mapping population lines by several QTL-parameters under strictly regulated conditions of the agroecological and biological testing area that depended on supplying of a nitrogen fertilizer (Table 1). For example, the duration of the periods, such as "seedlings–stem elongation," "seedlings–earing," "seedlings–flowering," and "seedlings–maturation," varied and depended on the introduction of nitrogen fertilizers. It is noteworthy that introduction of the fertilizers was repeated twice: the first time before sowing and the second at the stem elongation phase. The data obtained show that introduction of the fertilizers affected the estimation of location of the QTLs identified, which determined these four physiologically important traits of plant growth. Interestingly, the QTLs, which determine such periods as "seedlings–stem elongation," "seedlings–earing," and "seedlings–

Table 1. Traits and QTLs revealed in the ITMI mapping population under the regulated conditions of an agroecological and biological testing area in the absence (Experiment 1) and in the presence (Experiment 2) of nitrogen fertilizer

Trait	Symbol	Experiment 1**			Experiment 2**			Total*
		localization	LOD	R^2	localization	LOD	R^2	
Duration of the "seedlings–stem elongation"	VSB	5A (*89.0*)	5.78	22.04	5D (130.8)	3.38	13.30	**2** + 0 + 0
Duration of the "seedlings–earing"	VSH	5A (*89.0*)	3.43	14.23	2D (266.5)	2.60	15.90	**1** + 1 + 0
Duration of the "seedlings–flowering"	VSF	5A (*89.0*)	3.44	14.27	2D (266.5)	2.36	14.58	**1** + 1 + 0
Duration of the "seedlings–maturation"	VSM	2D (266.5)	3.09	18.91	7A (239.6)	2.69	29.34	**1** + 1 + 0
Plant height	PH	6D (*150.7*)	2.72	22.18	7D (136.7)	2.83	11.90	**0** + 4 + 0
		3A (*27.1*)	2.23	10.27	5A (*63.8*)	2.50	15.60	
Upper internode length	StLul	3A (*6.7*)	3.16	14.93	5A (*108.5*)	2.15	13.91	**1** + 1 + 0
Stem nod size	StNS	6A (88.8)	2.26	18.46	5D (130.8)	3.64	14.88	**1** + 1 + 0
Flag leaf length	LFL	4A (*124.4*)	2.96	12.63	1B (*178.9*)	2.61	16.62	**0** + 4 + 0
		6B (*111.4*)	2.14	16.99	5D (16.1)	2.53	10.58	
Flag leaf width	LFW	2D (*4.2*)	2.93	18.72	1B (*170.0*)	3.22	27.52	**1** + 3 + 0
		2A (*10.6*)	2.89	19.03	5A (*235.4*)	2.62	16.70	
Flag leaf pubescence	HLF	4B (*40.2*)	2.51	16.28	4B (112.1)	1.85	13.43	**0** + 1 + 1
Wax bloom on the internal side of leaves	LWBi	2D (*300.0*)	6.97	26.34	2D (*300.0*)	8.02	29.65	**2** + 0 + 0
Wax bloom on the external side of leaves	LWBo	2D (*300.0*)	22.92	63.40	2D (*300.0*)	23.63	64.53	**2** + 0 + 0
Wax bloom on the stem	StWB	2D (*300.0*)	22.69	63.03	2D (*300.0*)	22.40	62.56	**2** + 0 + 0
Wax bloom on the spike	SpWB	2D (*300.0*)	7.85	29.12	2D (*300.0*)	7.08	26.69	**2** + 0 + 0
Ligule color	LLC	7A (72.9)	2.21	13.87	7A (72.9)	1.81	11.56	**0** + 1 + 1
Auricle color	AuC	1A (*38.0*)	2.52	16.36	5B (96.3)	2.30	16.43	**0** + 5 + 1
		1B (*122.6*)	2.29	9.56	1A (*38.0*)	2.11	13.89	
		5B (96.3)	2.14	15.38	1B (*122.6*)	1.87	7.87	

Table 1. (Contd.)

Trait	Symbol	Experiment 1**			Experiment 2**			Total*
		localization	LOD	R^2	localization	LOD	R^2	
Spike texture	SpT	2A (*202.4*)	1.99	14.18	2A (*227.3*)	1.72	8.17	**0** + 0 + 2
Spike shape	SpS	1D (72.3)	2.79	17.44	1D (72.3)	2.12	13.55	**0** + 3 + 3
		5D (*194.3*)	1.83	11.49	7B (29.0)	2.01	13.63	
		7B (29.0)	1.73	11.90	5D (*194.3*)	1.82	11.41	
Spike fragility	SpBR	5D (3.7)	1.94	13.62	5D (5.1)	2.39	16.24	**0** + 1 + 1
Shape of the glume/flower chaff	GS	4D (*117.1*)	2.22	17.88	4D (*117.1*)	2.07	15.89	**0** + 2 + 2
		4B (*113.6*)	1.77	14.52	4B (*113.6*)	1.90	14.99	
Glume color	GC	1D (236.2)	3.23	14.65	1D (236.2)	3.47	14.25	**2** + 1 + 1
		7D (*59.0*)	2.78	18.95	7D (*59.0*)	1.69	11.12	
Grain color	KC	3B (131.7)	3.33	27.34	4A (*116.1*)	3.36	22.09	**2** + 1 + 1
		4A (*116.1*)	1.58	11.57	3B (131.7)	2.80	12.95	
Difficulty of thrashing	DifThC	5D (175.5)	2.15	15.42	5D (68.8)	2.50	16.21	**0** + 2 + 0
Spike length	SpL	4A (*102.8*)	2.60	11.83	1B (*182.7*)	3.10	12.84	**1** + 1 + 0
Number of spikelets per spike	NSpt	5A (*47.3*)	2.79	13.02	4A (*124.4*)	5.05	21.14	**1** + 1 + 0
Number of grains per spikelet	NSeSpt	4B (121.9)	2.94	19.33	1A (*144.1*)	2.63	10.99	**0** + 4 + 0
		2D (300.0)	2.63	12.34	7A (154.1)	2.27	19.57	
Number of grains per spike	NSeSp	2D (300.0)	3.56	16.31	7D (60.6)	3.57	27.12	**2** + 2 + 0
		7B (377.0)	2.47	16.75	3A (70.0)	2.83	12.21	
Weight of grains per spike	GMSp	2D (300.0)	4.25	19.17	1B (*229.3*)	2.56	18.13	**1** + 3 + 0
		7B (377.0)	2.17	14.92	7D (60.6)	2.23	17.93	

Table 1. (Contd.)

Trait	Symbol	Experiment 1**			Experiment 2**			Total*
		localization	LOD	R^2	localization	LOD	R^2	
Weight of 1000 grains	TGW	3B (146.5)	3.22	15.65	5D (*240.7*)	2.25	12.61	**1** + <u>1</u> + 2
		5D (130.8)	1.70	7.98	3B (245.6)	1.89	8.24	
Glassiness of grains	GrT	5D (*319.6*)	6.48	37.75	5D (*319.6*)	4.05	23.97	**4** + <u>0</u> + 0
		6A (49.1)	3.36	27.10	6A (49.1)	3.87	29.05	
Number of spikes	NStS	5A (*89.0*)	3.07	14.09	2A (*172.9*)	2.76	21.70	**1** + <u>3</u> + 0
		3D (*254.8*)	2.43	16.50	5D (140.5)	2.75	19.91	
Total*			**17** + <u>23</u> + 7			**14** + <u>25</u> + 8		**31**+<u>48</u>+15= 94

***** Bold font—main QTLs (LOD ≥ 3); <u>underlined</u>—strong QTLs (3 > LOD ≥ 2); regular font—minor QTLs (2 > LOD ≥ 1.5).
** Location of QTLs is shown in parentheses under the chromosome number; chromosomes are indicated by letter designations (*italic*—QTL is contributed by the maternal form Opata 85; regular font—QTL is contributed by the paternal form Synthetic). R^2—percentage of phenotypic variability determined by certain QTL.

flowering" were located strictly at the 89.0 cM position of the chromosome 5A before the introduction of nitrogen, while the "seedlings–stem elongation" trait was located in the chromosome 2D (130.8 cM) and the "seedlings–earing" and "seedlings–flowering" traits at the 266.5 cM position of the same chromosome after the introduction. The QTL that determine the duration of the "seedlings–maturation" period was located in the chromosome 2D (266.5 cM) before the introduction of a nitrogen fertilizer, though it was found in the chromosome 7A (239.6 cM) after the introduction. All four traits were identified at high confidence level. The LOD values varied from 3.09 to 5.78 in the experiments without nitrogen introduction and from 2.36 to 3.38 in the experiments with nitrogen introduction.

The plant height trait was instable. In the experiments without nitrogen introduction, this trait was determined by QTLs located in the 3A and 3D linkage groups, whereas it was located in the 5A and 7D linkage groups in the experiments with nitrogen introduction. In the experiments without nitrogen introduction, this trait was phenotypically expressed in 22.18% of cases, while it was expressed in 15.60% of cases in the experiments with nitrogen introduction. The localization of the QTLs identified for each of the traits of "upper internode length," "stem nod size," "the flag leaf length and width," and "the plant height," which is connected with them, was shown to be unstable. LOD values of these traits in the experiments without nitrogen introduction varied from 2.14 to 3.16, while it varied from 2.15 to 3.64 in the experiments with nitrogen addition.

Interesting data were obtained for the "flag leaf pubescence" trait. In both experiments, with and without nitrogen introduction, the QTLs revealed were located in the chromosome 4B, though in different regions. For example, in the experiments without nitrogen introduction, they were located almost in the very beginning of the linkage group in the position 40.2 cM, whereas they were found 71.2 cM farther in the experiments with nitrogen addition. This indicates that these two QTLs are different from one another. Contrastingly, the QTLs of the "wax bloom on the inner and outer leaf surface," as well as the wax bloom of the stem and ear, were found not only in the chromosome 2D but also in the position of 300.0 cM in both experiments. The QTLs of these traits are doubtlessly stable, because the LOD value and the phenotypic expression percentage were very high. For example, the LOD value of the "wax bloom on the inner and outer leaf surface" varied from 22.92 to 23.63, and the phenotypic expression percentage varied from 63.40 to 64.53 in both types of experiments. The QTLs of the ligule and leaf ear color were found in one and the same sites in the linkage groups 7A (ligule color), 1A, 1B, and 5B (ear color). The stability of the localization of the QTLs of these traits is doubtless. At the same time, the phenotypic expression percentage of these traits was quite high for this LOD assessment

and made up 13.87 and 11.56 for the "ligule color" trait and 2.14–2.52 and 1.87–2.30 for the "spike color" trait, respectively, at low and high agronomic background of nitrogen.

The "spike texture" trait, as well as the trait of the flag leaf pubescence, showed stable identification of the QTLs that determine them (2A). However, their localization varied and depended on the condition of whether the nitrogen fertilizer was supplied (202.4 cM) or not (227.3 cM). We believe that this trait, as well as the trait of the "flag leaf pubescence," was regulated by an individual QTL in each experiment.

Traits of "the shape and fragility of leafs," "the glume shape and color," and "the grain color" were determined by the same QTLs that were found in the same linkage groups and in the same positions for each of these traits in both experiments. The QTLs that determine the "spike fragility" were located in the chromosome 5D in both experiments. Difference in the location of this trait was 1.4 cM. This allows us to consider the chromosome locus that determines this trait to be the same in both experiments. The QTLs of the "difficulty of thrashing," which were also located in the chromosome 5D in both types of experiments, were found in the positions 175.5 cM (the experiment without the fertilizer) and 68.8 cM (the experiment with fertilizer). A difference of 106.7 cM, as well as relatively high LOD value, allow us to consider these two loci as independent ones, and to believe that manifestation of this trait depends on nitrogen introduction.

Traits that determine the yield structure, such as "spike length," "spike number," "the number of spikelets in the spike," "the number of grains in the spike and spikelet," and "the weight of grains obtained from one spike," were shown to depend on nitrogen introduction. The LOD value of these traits varied from 2.17 to 4.25 without nitrogen introduction and from 2.23 to 5.05 with nitrogen introduction. The phenotypic expression percentage varied from 11.83 ("spike length") to 19.33 ("the number of grains per spikelets") in experiments without nitrogen, and from 12.21 to 27.12 (both indicators of the "number of grains per spike") in experiments with nitrogen introduction.

Another trait of the crop yield structure is the "weight of 1000 grains." It was characterized by stable localization of QTLs in the linkage groups (3B and 3D). However, the localization of the QTLs identified differed significantly depending on nitrogen supplementation. For example, for the linkage group 3B, the QTLs were found in the position 146.5 cM (the experiment without nitrogen supplimentation) and in the position 246.5 cM (the experiment with nitrogen supplimentation), while they were found in the position 130.8 cM (the experiment without nitrogen supplimentation) and in the position 240.7 cM (the experiment with nitrogen supplimentation) for the linkage group 5D, indicating them as different loci within the same chromosomes. Therefore, the trait of the "weight of 1000 grains," as well as all other yield structure traits analyzed in our study, depends on nitrogen supplying. This obviously points at the importance of nitrogen for physiological and genetic expression of the traits that determine the yield structure of such an agriculturally valuable culture as wheat.

The trait of "grain glassiness" was expressed stably in both types of experiments. High values of LOD and the phenotypic expression percentage confirm the stability of its manifestation and allow us to suggest that introduction of a nitrogen fertilizer has no effect on the activity of genetic determinants, which determine physiological parameters of "grain glassiness."

The correlation analysis, which was additionally carried out, revealed traits whose manifestation varied and depended on nitrogen supplimentation (Table 2). It was shown that the yield structure traits positively correlated with doses of nitrogen supplied (Table 2): "spike length" ($r_{xy} = 0.59$), "number of spikes" ($r_{xy} = 0.60$), "number of spikelets per spike" ($r_{xy} = 0.59$), "number of grains per spike" ($r_{xy} = 0.52$), "number of grains per spikelet" ($r_{xy} = 0.31$), and "weight of grains in the spike" ($r_{xy} = 0.54$). Traits of initial growth and development poorly correlated with nitrogen supplying: the duration of the "seedling–stem elongation" period ($r_{xy} = 0.20$), the duration of the "seedling–earing" period ($r_{xy} = 0.28$), the duration of the "seedling–flowering," period ($r_{xy} = 0.31$) and the duration of the "seedling–maturation" period ($r_{xy} = 0.16$).

Traits of the "upper internode length" and the "inter nod size" demonstrated poor ($r_{xy} = 0.16$) and medium ($r_{xy} = 0.46$) positive correlations and depended on the dose of nitrogen supplied. The trait of the "plant height" also demonstrated medium positive correlation ($r_{xy} = 0.37$), which is consistent with the data of the analysis of variances and the QTL-analysis.

These conclusions were confidently confirmed by the data of single-factor analysis of variance on the effect of the factor on the trait (Table 3). The statistical analysis revealed that 15 of 31 traits depended on supplying of the nitrogen fertilizer. According to the common rules [25], the confidence level of $P < 0.05$ shows that traits with these parameters of confidence varied as strong as close to zero the confidence level. At the same time, four traits, "the flag leaf pubescence," "spike texture," "difficulty of thrashing," and "the weight of 1000 grains," which demonstrated different chromosomal localization in the QTL and other statistical analyses but not the difference in the linkage group in the single-factor analysis of variance, did not show a statistically significant connection with the level of nitrogen. The other 12 traits analyzed, the p-value of which in the dispersion ratio (F) was higher of equal to 0.05, were stable, demonstrating physiological and genetic independence on the level of nitrogen.

On the whole, the data of the single-factor analysis of variance confirm the results of the correlation and

Table 2. Data of the correlation analysis

Trait*	r_{xy}—correlation coefficient	*t*-test	*p*-confidence
VSB**	0.20	2.95	0.003
VSH**	0.28	4.33	0.000
VSF**	0.31	4.74	0.000
VSM**	0.16	2.43	0.016
PH**	0.37	5.68	0.000
StLuI	0.16	2.30	0.023
StNS**	0.46	7.26	0.000
LFL**	0.61	11.26	0.000
LFW**	0.60	11.13	0.000
HLF	0.06	0.82	0.414
LWBi	0.01	0.17	0.868
LWBo	0.02	0.32	0.747
StWB	–0.01	–0.09	0.931
SpWB	–0.05	–0.67	0.506
LLC	–0.01	–0.18	0.858
AuC	0.00	0.00	1.000
SpT	–0.04	–0.63	0.528
SpS	0.00	0.00	1.000
SpBR	–0.11	–1.46	0.146
GS	0.02	0.24	0.807
GC	–0.10	–1.38	0.168
KC	0.10	1.48	0.140
DifThC	–0.03	–0.40	0.686
SpL**	0.59	10.21	0.000
NSpt**	0.59	10.24	0.000
NSeSpt**	0.31	4.63	0.000
NSeSp**	0.52	8.50	0.000
GMSp**	0.54	9.00	0.000
TGW	0.09	1.26	0.210
GrT	–0.03	–0.49	0.628
NStS**	0.60	10.68	0.000

* Abbreviations of the traits are the same as in Table 1.
** Traits characterized by statistically confident variability, which depended on the conditions of growth (with or without nitrogen fertilizer).

the QTL analyses, showing the significance of effect of nitrogen fertilizers on growth, development, and formation of productivity traits in spring soft wheat under strongly regulated conditions of agroecological testing area.

DISCUSSION

QTL mapping provides the possibility to identify not only individual loci of individual linkage groups, which are involved into realization of a trait, but also a tool to discriminate the manifestation of a trait in different ecological and geographical regions of the world or under different conditions of growing. This refers to such important physiological and genetic traits as consumption of nitrogen fertilizers supplied at different stages of plant growth and development. The increase in the nitrogen consumption efficacy, especially by cereals, is one of the most challenging problems of agricultural productivity. Plants that assimilate nitrogen more effectively may produce better yield with increased level of protein of better quality. Nowadays, we can reach this goal only through identification of physiological and genetic mechanisms of plant productivity and improvement of the physiological and genetic structure of a plant genotype. The latter may

Table 3. Data of the single-factor analysis of variance*

Trait**	Number of degrees of freedom d.f.	Standard deviation MS	*F*-dispersion ratio	*p*-confidence	Remaining variation (error)	
					d.f.	MS
VSB***	1	387.56	8.72	0.003	218	44.44
VSH***	1	2294.05	18.78	0.000	217	122.14
VSF***	1	1452.06	22.43	0.000	213	64.75
VSM***	1	582.98	5.88	0.016	212	99.11
PH***	1	2972.32	32.29	0.000	199	92.06
StLuI***	1	102.96	5.28	0.023	199	19.48
StNS***	1	0.07	52.75	0.000	199	0.00
LFL***	1	1192.72	126.68	0.000	215	9.42
LFW***	1	2.05	123.98	0.000	215	0.02
HLF	1	1.51	0.67	0.414	210	2.26
LWBi	1	0.07	0.03	0.868	214	2.69
LWBo	1	0.46	0.10	0.747	214	4.43
StWB	1	0.07	0.01	0.931	216	9.63
SpWB	1	1.85	0.44	0.506	214	4.18
LLC	1	0.00	0.03	0.858	216	0.14
AuC	1	0.00	0.00	1.000	216	0.47
SpT	1	0.46	0.40	0.528	216	1.15
SpS	1	0.00	0.00	1.000	216	0.29
SpBR	1	0.43	2.13	0.146	186	0.20
GS	1	0.04	0.06	0.807	199	0.59
GC	1	8.88	1.91	0.168	198	4.64
KC	1	0.51	2.20	0.140	198	0.23
DifThC	1	0.43	0.16	0.686	198	2.61
SpL***	1	148.40	104.15	0.000	199	1.42
NSpt***	1	549.78	104.77	0.000	199	5.25
NSeSpt***	1	8.93	21.43	0.000	199	0.42
NSeSp***	1	6868.42	72.22	0.000	199	95.11
GMSp***	1	147.88	80.99	0.000	198	1.83
TGW	1	76.70	1.58	0.210	196	48.49
GrT	1	0.10	0.24	0.628	200	0.41
NStS***	1	240.80	114.04	0.000	198	2.11

* Factor—variation of experiment.
** The abbreviations of the traits are the same as in Table 1.
*** Traits characterized by statistically confident variability, which depended on the conditions of growth (with or without nitrogen fertilizer).

be achieved through identification of genes and loci that regulate these mechanisms.

To date, there several studies that identified the QTLs of nitrogen consumption by hexaploid wheat (*Triticum aestivum* L.) under both regulated [5–7] and field conditions [8–10]. There are also studies that revealed individual genes and genomic elements involved in the assimilation of mineral nitrogen [10, 12, 26]. However, it is common knowledge that different accessibility of NO_3^- and NH_4^+ in soil is one of the most important factors of mineral nitrogen assimilation failure. Moreover, both season and daily changes in growth and other physiological processes cause gradient needs in nutritive substances that come from soil. Therefore, we estimated the location of QTLs responsible for agronomically valuable traits determined by the

nitrogen assimilation efficacy (mineral fertilizers introduced into soil) in spring soft wheat (*T. aestivum* L.) in the linkage groups at different doses of nitrogen under the conditions of North-West Russia [27].

It is noteworthy that, in this experiment, QTLs were revealed under common field conditions, in which the key abiotic factors that determine plant growth and development were not regulated. It was shown that specific QTLs of the productivity traits demonstrated higher physiological and genetic activity at low doses of nitrogen, and may be, therefore, used to provide the stability of yield via combination of QTLs that determine these traits and manifest under different ecological conditions at a low level of mineral nitrogen.

Experiments of other scientists revealed the genetic variability of the "crop productivity" trait at a low level of nitrogen. It was shown that physiological and genetic interaction between the genotype and the level of nitrogen is important for realization of physiological and genetic capacities of plants [28]. The same authors showed that nitrogen assimilation basically explains variability in the mineral nitrogen consumption efficacy at a low level of nitrogen as well as its physiological importance for the "crop productivity." Moreover, it was found that direct selection at low levels of nitrogen fertilizers is most effective for wheat. Other studies carried out of the doubled haploid lines also confirmed the effectiveness of direct selection at a low level of nitrogen fertilizers as well as at the gradient access of soil nitrogen [5]. Moreover, French scientists showed that specific QTLs of the productivity traits identified under low level of nitrogen fertilizers may be used to obtain stable yields, because of a combination of QTLs connected with these traits and that manifest at different ecological conditions at low levels of mineral nitrogen [8].

However, it is noteworthy that, in all studies mentioned above, the QTL-analysis was performed either under common field conditions typical for an ecological region, without limitations of soil barriers similar to those used in our study [27], or under the conditions of hydroponic culture, or vegetation pots under greenhouse conditions [5–7]. These conditions were principally different from the agroecological and biological testing area, which allow studying a number of plants under the same strongly regulated conditions [14].

The main feature of a regulated agroecosystem is the possibility to modify individual components of the environment without changing the other factors that affect vegetative plants. This provides preconditions for the following precise analysis of physiological and genetic activity of the quantitative trait loci identified and exploration of the "QTLs–environment" interaction under natural conditions of growing in comparison with a regulated agroecosystem.

In the present study, we for the first time revealed the linkage group localization of the QTLs that control 31 quantitative traits of hexaploid wheat under strongly controlled conditions of a agroecological and biological testing area with and without introduction of nitrogen fertilizer. QTLs of 19 traits assessed at different concentrations of accessible nitrogen changed their localization in the linkage groups. This is, apparently, due to the character of the "genotype–environment" interaction. For example, although localization of wax bloom QTLs in chromosomes remained the same in variations of experiments, the QTLs of productivity, conversely, were unstable and changed their location in the linkage groups depending on the presence of nitrogen fertilizer. Apparently, this is due to realization of the adaptive potential of plants under certain conditions of growing [15, 27, 29]. It appears that a complex effect of environmental factors determines the specific features of the coadaptive gene blocks of each plant species, including wheat, formed during evolution and coadaptation specificity of its whole genetic system. It is known that both evolutionary and ontogenetic "memory" of the genetic systems, *F* and *R*, are formed on the same basis [29].

On the whole, all QTLs identified (Table 1) may be conditionally divided into two groups: dependent and independent on the introduction of nitrogen fertilizers, i.e., on the targeted influence of the environment. The data of QTL showed that the group of depended QTLs involved all chromosomal loci that were in any way involved in the regulation of plant productivity, whereas the group of independent QTLs included traits on which the introduction of nitrogen fertilizers produced no effect. Moreover, the QTL-mapping revealed the distribution of the identified loci among the linkage groups. For example, the main QTLs of the duration of the "seedlings–stem elongation," "seedlings–earing," "seedlings–flowering," and "seedlings–maturation" traits in experiments without nitrogen supplimentation formed the 2D and 5A clusters, while they formed the 2D, 5D, and 7A clusters in experiments with nitrogen supplying. Traits of the wax bloom on the internal and external sides of leaves, as well as on the stem and ear, formed a single cluster on the 2D chromosome in both experiments, regardless of nitrogen supplying.

Conversely, the formation of the linkage group clusters of the crop yield traits depended on the condition of whether the nitrogen fertilizer was supplied or not. For example, in experiments without nitrogen supplying, the QTLs of the traits of the "spike length," "number of spikes," "number of spikelets per spike," "the number of grains per spike and per spikelet," and "the weight of grains per spike" were located in the 2D, 4A, 4B, 5A, and 7B chromosomes, respectively, while they were located in the 1A, 1B, 3A, 4A, 7A, and 7D linkage groups, respectively, in experiments with nitrogen supplying.

Another productivity trait is "the weight of 1000 grains," which formed two clusters in the chro-

mosomes 3B and 5D in both types of experiments. However, the clusters found in these linkage groups demonstrated different localization depending on supplying of nitrogen. On the whole, the results of our study are well consistent with the data obtained previously in our studies [16] as well as by Chinese [11] and French researchers [12], who revealed QTLs that determined the efficacy of mineral nitrogen fertilizers with respect to productivity and its components in almost the same linkage groups: for example 2D, 4B, 5A, and 7A. Nevertheless, it is noteworthy that the traits of "the grain weight" and "the number of grains per spike" are usually located in the same chromosomes, whereas the trait of "the weight of 1000 grains" is controlled by the QTL, whose location depends on environmental factors. Under the regulated conditions of agrobiological testing area it was located in the chromosomes 3B and 5D, while it was identified in the chromosomes 5B, 6D, and 7D in open soil, which is characterized by variable temperature and humidity [27].

We cannot compare the results of the present study with the data of previous investigations, which were also obtained under the regulated conditions of agrobiological testing area [14], because the designs of the experiments differed significantly. The main difference between these experiments was connected with the nutrition regime of plants. In the previously published paper [14], plants were grown on peat supplemented with chalk, clay, and superphosphate. Watering was interchanged by the addition of Knop's solution, which contained microelements. In the present study, plants were grown on sod-podzol soil, which contained a rather high level of nitrogen, phosphorous, and potassium. In the experiments with high level of nitrogen nutrition, plants were supplemented with amide nitrogen.

It is noteworthy that the specific feature of an agroecological system is that it allows separating of individual components of the environment without changing other factors, which affect vegetating plants. The expression of QTLs identified in the regulated agroecological system may either depend or not on the factor studied. In contrast to the previous experiments, in which temperature and lighting regimes varied, all growing conditions except the level of nitrogen were constant in the present study [14]. We did not pursue the goal to compare the effect of growing conditions in two different series of the experiments on identification and localization in the linkage groups of the chromosomal loci that are involved in the manifestation of the traits studied. It is noteworthy that seeds used in the study were obtained under different soil and climatic conditions, and the root-inhabited environments in these two series of experiments were also different. At the same time, it is noteworthy that traits of the "seedlings–stem elongation," "number of spikes," "number of cones per spike," and "the weight of grains per spike" obviously depended on both temperature and light regimes and on the presence of nitrogen fertilizer. We also identified the traits of "the wax bloom on the internal side of leaves," "the wax bloom on the external side of leaves," and "the fragility of leaves," the manifestation of which varied and depended on temperature and lighting but did not depend on the dose of mineral nitrogen.

Apart from the QTL-analysis, the correlation analysis and the single-factor analysis of variance were carried out in order to confirm the statistic connection between the traits and the variations of the experiments (Table 2, 3). It should be noted that, in case of QTL-analysis, the stability of trait identification in the linkage groups of the chromosomal loci, which determine the expression of the trait studied, was also assessed. Moreover, it was necessary to confirm the significance of the connection between the values of interest and to make sure that the factor (variation of the experiment) significantly affected the trait. The first task was solved by the correlation analysis, and the second was solved by the dispersion analysis. All statistical approaches, including the QTL-analysis, in the present study, demonstrated almost the same results except four traits: "the flag leaf pubescence," "spike texture," "difficulty of thrashing," and "the weight of 1000 grains." This is, apparently, due to the use of the same original variants in all three statistical methods, as well as to use of the maximum likelihood criterion and assessment of the statistic confidence in each mathematical approach. The main difference between these approaches is that, in the QTL-analysis, the threshold confidence level of LOD value was set in the very beginning, while the statistic confidence level was calculated individually for each trait in the single-factor analysis of variance and in the correlation analysis. On the whole, the results of the mathematical calculations used to analyze the traits of the "flag leaf pubescence," "spike texture," and the "weight of 1000 grains" showed that the algorithm of the QTL-analysis is advantageous as compared with the single-factor analysis of variance and correlation analysis, taking into account the experimental material used, i.e., the recombinant inbred lines of the ITMI mapping populations. This allows us not only to identify the positions of the QTLs responsible for manifestation of a trait and to calculate the percentage of phenotypic variability determined by each of the QTL identified but also to reveal the molecular markers [19], which are genetically linked to the QTLs identified, because lines of the mapping population are saturated with molecular markers [14–16, 19, 27]. This information allows us not only to make calculations and assess their confidence but also reveal the localization of the QTLs identified even in the same chromosomes.

Therefore, in the present study, we have demonstrated for the first time the dependence of physiological activity of the genetic determinants that determine the expression of agronomically important quantita-

tive traits of hexaploid wheat on the dose of nitrogen fertilizers supplied under strongly regulated growing conditions. The data obtained allowed us to reveal the genomic regions involved in the metabolism of nitrogen, including the traits of growth and productivity of spring soft wheat. This may provide more accurate identification of chromosome loci, especially those connected with identification and practical transfer of the allelic variations of genetic determinants responsible for expression of agriculturally valuable physiological traits. In the future, it may allow us to estimate individual stages of physiological and genetic mechanisms and their realization.

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