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The content of free amino acids and water-soluble carbohydrate in leaves of isogenic by VRN gene lines of wheat.

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Abstract. In a three-year experiment, we observed the effects of *VRN* genes in soft wheat on the development rate (shoot–booting period), content, and distribution of free amino acids and water-soluble carbohydrates in isogenic lines of Mironovskaya 808 and Olviya. Slowly developing isolines are characterized by a lesser amount of free amino acids and monosaccharides in the leaves. The correlation index C/N clearly correlates with the rates of the isoline development. We propose that *VRN* genes are involved in synthesis control, composition, and distribution of the basic photoassimilates of soft wheat.

Introduction. The transition of wheat plants from vegetative to generative is a major stage in the ontogeny, determining many economically valuable traits. The key genes in this process are the *VRN* genes, which define the reaction of wheat to vernalization and, thus, determine the rate of development. The reaction to vernalization in wheat is controlled by at least five genes, three of which, *Vrn-A1a, Vrn-B1a,* and *Vrn-D1a*, are localized on chromosomes 5A, 5B, and 5D, respectively. Winter-type plant development is manifested only when all three genes are recessive. If at least one of these genes is dominant, then the plants are spring type. *VRN* genes have been actively researched at the molecular-genetic level (Loukoianov A et al. 2005; Oliver SN et al. 2009) and have been cloned and several allelic variants for wheat described (Preston JC et al. 2009). Genes *Vrn-A1a* and *Vrn-B1a* are transcription factors (Distelfeld A et al. 2009; Trevaskis B 2010). These studies have deepened the notion of genetic and molecular-biological control of wheat development.

The primary products of photosynthesis are soluble carbohydrates and free amino acids. Their content, correlation, and distribution between the main organs, in many ways, define the rate of plant development, productivity, and protein content in grain. At present, nitrogen and carbonic metabolism of cereals is intensively studied, including the distribution of metabolites in plant organs and aspects of their reutilization of photoassimilates during grain formation (Amane M 2011), the connection between photosynthesis and general productivity (Champigny M-L 1995), and the increase of valuable proteins in grain (Marte P et al. 2003). Coruzzi and Bush (2001) have shown that low-molecular-weight nitrogen and carbohydrate compounds in plants in nature are not just metabolites, but signal molecules that activate and/or repress many genes.

Perhaps, the *VRN* genetic system, which determines the rate of plant development, reveals the mediated effects on the content of basic metabolites (photoassimilates) of the whole plant, their distribution to the organs of the main shoot, and the correlation of carbohydrate and nitrogenous metabolites. Our investigation researched the content and distribution, by plant organ, of the carbohydrates and amino acids in connection with the rate of wheat development.

Materials and methods. A proper study of the physical-biochemical process is possible only using near isogenic lines (NILs), which have minimal differences in all the genes besides the marked specific feature. We used NILs that were monogenic dominant for the *VRN* genes of soft wheat, which differed in their rate of development. These lines were created in the background of winter wheat cultivars Mironovskaya 808 (tall) and Olviya (short). Field experiments were made during 2010–12 at experimental plots of the Department of Physiology and Biochemistry, V. N. Karazin Kharkov National University. Observations were made on the duration of the shoot-to-boot period and biochemical analyses of the content of free amino acids in the different plants organs and the fractional composition of reduced saccharides in the leaves during the of booting–flowering period.

Results and discussion. The distribution of free amino acids in the organs of the main shoot in the NILs is at a maximum in the leaves, the main photosynthetic organ. Sufficiently high amine nitrogen content was revealed in forming spikes, which become the major sink during flowering and accept both nitrogenous and carbohydrate photoassimilates. The least amount of free amino acids was in the stem, perhaps because it is mainly for transport does not take part in metabolic processes. Such a distribution of free amino acids by the organs of main spike is typical for tall (Mironivskaya 808) and short (Olviya) NILs. However, we noted that Olviya NILs had a higher content of free amino acids in the forming spikes, which may be explained by its faster development compared to that of the taller Mironovskaya 808.

A clear influence of NIL genotype on the content of free amino acids is revealed only in the leaves. In both cultivars, slow-developing NILs, with *VRN-B1a*, were characterized by the minimum content and quick-developing NILs, with *VRN-A1a* and *VRN-D1a*, were characterized by the maximum.

The level of amino acids in the spikes of slowly developing, *VRN-B1a* of Olviya was higher than that of the spikes of the quicker developing, *VRN-A1a* and *VRN-D1a* Olviya lines (Table 1), which may be connected with a slower amino acid synthesis of protein in the forming grain in slowly developing line compared with rapidly developing lines.

Table 1. The distribution of free amino acids in the organs of the main shoot of *VRN* (dominant gene) NILs of wheat.

Cultivar	Isoline genotype	Amino acid content (mg/g dry mass (mean ± σ)		
		Leaf	Stem	Spike
Mironovskaya 808	*VRN-A1a*	1.39 ± 0.06	0.74 ± 0.06	0.99 ± 0.02
	VRN-B1a	1.21 ± 0.03	0.57 ± 0.02	0.53 ± 0.01
	VRN-D1a	1.32 ± 0.05	0.55 ± 0.07	0.77 ± 0.02
Olviya	*VRN-A1a*	1.37 ± 0.04	0.66 ± 0.07	0.46 ± 0.01
	VRN-B1a	1.08 ± 0.02	0.65 ± 0.04	1.95 ± 0.02
	VRN-D1a	1.34 ± 0.05	0.63 ± 0.02	1.33 ± 0.05

Water-soluble carbohydrates, the main products of photosynthesis, are represented in plants mainly by glucose, fructose, and the disaccharide saccharose. The fractional composition of water-soluble carbohydrates in leaves of isogenic by genes *VRN* lines of wheat (Table 2) showed that monosaccharides predominate and make up 60–80% of the synthesized unstructured carbohydrates in leaves. The content of the different fractions (mono-, oligo-, and total) of water-soluble carbohydrates in leaves of both the tall Mironovskaya 808 and short Olviya were virtually the same. The level of monosaccharides in the leaves correlates with the rate of isoline development. The slow-developing *VRN-B1a* isoline is characterized by a minimal content, and rapidly developing *VRN-A1a* and *VRN-D1a* lines by the maximum. We also found the same results while investigating free amino acids in the leaves (Table 1). The amount of oligosaccharide that is present as the main transport carbohydrate in plants, saccharose, is the minimum in isolines that are the first the flower among isolines of Mironovskaya 808 with *VRN-D1a* and among isolines of Olviya with *VRN-A1a* (Table 2). Maybe, the spikes, which are greater sinks, have faster rates of photoassimilates for synthesis of reserve carbohydrates and proteins during grain formation.

Table 2. The content of water-soluble carbohydrates in leaves isogenic by *VRN* gene in NILs (dominant gene). of wheat.

Cultivar	Isoline genotype	Content of soluble carbohydrates, mg/g dry mass (mean ± σ)		
		Mono-	Oligo-	Total
Mironovskaya 808	*VRN-A1a*	37.6 ± 0.06	15.6 ± 0.06	53.2 ± 0.02
	VRN-B1a	36.5 ± 0.03	15.2 ± 0.02	51.7 ± 0.02
	VRN-D1a	45.3 ± 0.05	8.6 ± 0.07	53.9 ± 0.02
Olviya	*VRN-A1a*	38.0 ± 0.04	13.9 ± 0.07	51.9 ± 0.02
	VRN-B1a	36.2 ± 0.02	19.0 ± 0.04	55.2 ± 0.02
	VRN-D1a	38.7 ± 0.05	16.3 ± 0.02	55.0 ± 0.02

Flowering is the determiner for forming the future yield. Photosynthetic activity and nitrogenous status influence the dry mass and content of protein in grain (Champigny M-L 1995; Lawlor DW 2002). The duration of the shoot-to-flowering period showed that the NILs are clearly divided into slowly and rapidly developing groups (Table 3). Independent from the general duration

Table 3. The duration of the shoot-to-flowering period and the correlation of C/N in leaves of isogenic *VRN* lines of wheat (mean ± σ).

Cultivar	Isoline genotype	Shoot-to-flowering (days)	C/N
Mironovskaya 808	*VRN-A1aB1bD1b*	57 ± 1	38.27 ± 1.2
	VRN-A1bB1aD1b	65 ± 2	42.73 ± 1,8
	VRN-A1bB1bD1a	52 ± 1	40.83 ± 1.6
Olviya	*VRN-A1aB1bD1b*	47 ± 1	37.88 ± 0.9
	VRN-A1bB1aD1b	61 ± 2	51.11 ± 2.1
	VRN-A1bB1bD1a	50 ± 1	41.04 ± 1.5

of the transition time to the generative stage that is defined by genotype, individual *VRN* genes in both cultivars slow down or speed up the isoline development. Slowly developing isolines have the *VRN-AlbBlaDlb* genotype and quickly developing isolines are *VRN-AlaBlbDlb* and *VRN-AlbBlbDla* genotypes. In the field conditions of natural photoperiod at latitude 50° Kharkov, the Mironovskaya 808 NIL with *VRN-AlbBlbDla* was the first to flower followed by the Olviya NIL with *VRN-AlaBlbDld*.

The correlation of carbohydrate and nitrogen levels serves as indirect balance index of the carbohydrate and nitrogen metabolism of plants. We calculated the correlation of carbohydrate and free amino acid content. The correlation index, C/N, in leaves had a maximm value in NILs that were characterized by the longest period of days-to-heading, i.e., the slowly developing NILs, which can be evidence for dependence of carbohydrate and nitrogen metabolism on genotypes of *VRN* genes.

We found out that individual *VNR* genes, and maybe genetic systems in general, which regulate the rate of plant development in wheat, indirectly determine the amount, composition, and distribution between the organs of the main photoassimilates, water-soluble carbohydrates and free amino acids. Slowly developing NILs are characterized by a lower amino acid and monosaccharide content in the leaves during flowering, which possibly can be connected with less productivity of photosynthetic processes in these isolines. The C/N correlation index clearly agrees with the rate of development in the NILs. These effects do not depend on the genotype of the cultivar, but appear in NILs irrespective of their cultivar peculiarities.

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Chemical protection of winter wheat shoots using a presowing seed treatment.

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In the chemical protection of winter wheat, especially at the first stages of organogenesis, a presowing seed treatment is ecologically safe for the environment, technologically easy, and economically profitable. Lately, among the assortment of insecticide treatments, neonycotynoids are obtaining wide application.

Our investigation studied the phytosanitary role of chemical treatment of winter wheat seed with systemic insecticides, especially from the neonycotynoid, group for reducing pest injury, and, to increase grain yield.

All studies were conducted at the Laboratory for Plant \Pproduction and Cultivar Investigations of Plant Production Institute nd. a. V.Ya. Yuriev (Eastern Forest-Steppe of Ukraine) during 2008–12. The soil was a typical medium-humus black earth soil on loess with humus with a plowing layer to 5.4%. Seed of winter bread wheat were pretreated with a tank preparation mixture of insect-fungicid. The active agent, imidaclopryd, was 0.25 kg/t and 0.35 kg/t of seed. Winter wheat was sown at optimal dates with the following rates: 4.0 x 10^6 viable seed/ha on black fallow and 5.0 x 10^6 viable seeds/ha after dried peas. Winter wheat was sown in two blocks, without fertilizer and with and application of organic-mineral fertilizer (humus, 6.7 t/ha of crop rotation area and (NPK) 30–60). The agronomic techniques employed are widely used in the investigated area. Counts for plant damage by the larvae of flies were according to conventional methods.

Results. Insecticide treatments, on the basis of imidaclopryd, was highly effective against intrastalk pests, namely fly larvae (Table 1). Averaged over two years, the effectiveness of a pretreating winter wheat seed with imidaclopryd prior to sowing was 78.3%, including *Oscinella* (Diptera: Chloropidae), 85.2%; *Mayetiola destructor* (Diptera: Cecidomyiidae), 66.7%; and *Phorbia securis* (Diptera: Anthomyiidae), 62.5%.

Table 1. Carbon isotope ratio and carbon concentration in leaves of six cover crops grown in Manhattan, KS, and harvested in 2011 (mean of eight values + SE).

Cover crop	δ^{13}C ($^o/_{oo}$)	C ($^o/_o$)
Winter wheat	–27.31±0.15	34.41±2.71
Triticale	–29.02±0.29	29.73±4.95
Oat	–29.07±0.10	31.92±2.05
Austrian winter pea	–29.52±0.65	39.70±0.87
Red clover	–30.48±0.16	41.93±4.64
Alfalfa	–31.35±0.51	39.36±0.74

During the autumn tillering in 2010, solitary damage to plants by flies was observed, so observations were not made at this time. In autumn 2011, a drought resulted in a majority of seed sprouting 30–35 days after sowing. The plants finished the autumn vegetation at the two–three leaf stage. Again, counts for plant damage by pests during this period were not carried out. In the winter wheat fields during autumn tillering in 2012, the dominant flies were *Oscinella spp*. These larvae caused 25.1% shoot damage in the control and 8.8% shoot damage in the (fungicide+insecticide). Chemical treatment of winter wheat seeds with imidaclopryd (0.35 kg/t seeds) has shown to be effective against *Oscinella* larvae (36.4%). The lowest level of damage to the shoots with *P. securis* and *M. destructor* larvae were 0.9% and 0.2%, respectively. According to the 2012 data, imidaclopryd was highly effective against soil pests. The technical effectiveness of the preparation against larvae (Coleoptera: Elateridae) with a dose 0.25 kg active agent/t of seed was 87.5% and 100% at 0.35 kg/t.

Pretreating winter wheat seed prior to sowing with a tank mixture of fungicides and insecticides (on the basis of imidaclopryd) is economical profitable, especially in fertilized blocks (Table 2). Averaged over 2009–12, the increase in the grain yield from applying tank mixtures of fungicides and insecticides (imidaclopryd) was 0.24 t/ha, ranging from 5.25 t/ha (control, without chemical protection) to 5.49 t/ha. Seed pretreatment with this mixture in the block with organic-mineral fertilizers contributed to the increase in grain yield by 33.9% (from 3.63 t/ha, control, without chemical protection and fertilizers, to 5.49 t/ha).

Table 2. Economical effectiveness of pre-sowing seed treatment of winter bread wheat with insecticide–fungicide compositions during autumn tillering in 2008–09.

	Yield capacity (t/ha)				Yield increase (t/ha) from	
Variant	2009	2011	2012	2009–12 average	chemical protection	chemical protection + fertilizers
Control (without fertilizers)	3.18	4.80	2.90	3.63	—	—
Control (organic - mineral block)	5.67	6.75	3.34	5.25	—	—
Fungicide + insecticide with active agent imidaclopryd	6.04	6.90	3.53	5.49	0.24	1.86