Analysis of the effect of the *sdw1/denso* gene nucleotide polymorphism on the variability of the main economically valuable traits of winter barley

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Abstract

The improvement of lodging resistance is one of the main tasks in winter barley breeding. It has been established that lodging resistance is associated with plant height. At present, the use of the semidwarf gene *sdw1/denso* in the breeding process is of great importance in the development of modern varieties. The semidwarf varieties with the gene *sdw1/denso* mutations are widely spread among the sowings of short-stem barley fodder varieties in the western states of US, in Canada, Australia and among spring brewery varieties in Europe. The varieties with the *sdw1/denso* gene are included in the genealogy of most modern barley varieties. The *sdw1/denso* gene has a pleiotropic effect, that is, it affects many traits, including the general adaptability and seed productivity of plants. In this regard, the purpose of the work was to compare the allelic polymorphism of the *sdw1/denso* gene with the results of the phenotypic assessment of economically valuable traits of winter barley. It was established that the *sdw1/denso* gene alleles are reliably associated with 'grain size (coarseness)' as one of the main parameters of yield. Thus, the dominant (mutant) allele of the *sdw1/denso* gene identified with the used CAPS-marker, can be recommended for selection of more productive genotypes.

Keywords: winter barley, lodging resistance, nucleotide polymorphism, gene, grain size (coarseness).

Improvement of grain production and qualitative traits are the main tasks of agricultural producers. Barley is a universal grain crop, both in terms of its distribution and its versatile use (Filippov et al., 2012). Barley grain, especially grown in the southern and southeastern arid regions, is an unsurpassed grain culture in its nutritional value. One kg of barley contains 100 g of digestible protein and 1.28 of feed units. This is more than in oats and rye. An extremely important feature is that barley possesses a full set of essential amino acids. The use of barley as a component of animal feed contributes to the improvement of farm animals' productivity (Alabushev et al., 2018).

Since the barley yields considerably vary throughout the years, one of the main issues of modern agricultural production is the stabilization of grain production, regardless of changes in weather conditions (Batalova, 2015).

At the present stage of agricultural development, it is the variety that is the most effective and most affordable means to improve the yield amount and quality, to save energy, to increase profitability and competitiveness of agricultural production (Shevtsov et al., 2007).

Winter barley is one of the most productive grain crops. Occupying about 5% of the total area of barley sowing in Russia, it annually produces up to 10% or more of the gross grain yield. However, its potential is not fully realized due to the insufficient strength of the straw; as a result, in the years favorable for growth and development of plants, barley often lays down, which results in a significant yield decrease (Shevtsov, 1982). One of the reasons for the yield decrease is the difficulty of mechanized harvesting, and therefore, the varieties resistant to lodging are of particular importance (Kovtun, 2002).

The solution of the problem of barley lodging resistance is associated, first of all, with plant height. In the Rostov region, when heavy rains are followed by acute droughts, the problem of plant height acquires a very special significance. Tall-grown barley varieties affected by rains with winds are significantly inclined to lodging (Filippov et al., 2010).

Lodging reduces not only barley yields and grain quality, but also indirectly affects malt quality, since the grain obtained by threshing lodged plants has a less 1000-grain weight and worse qualitative traits (Day et al, 1958). Due to it, breeding of lodging resistant barley varieties has always been of great importance.

The phenomenon of plant lodging has been studied for more than two centuries. At first, lodging resistance was associated with plant height. And the first successes were achieved by reducing the height of the plants. So, in the P.P. Lukyanenko National Center of Grain the use of the erectoid morphotype in breeding programs allowed reducing the height of winter barley plants on 20-30 cm, which resulted in lodging resistance improvement, and to a significant yield increase (Shevtsov, 1982).

The recent achievements in the grain crop genomics allowed to analyze the architectonics of the grain genomes, which contributed to the accumulation of knowledge about the genes associated with the main agronomic traits. Currently, the use of the semi-dwarf gene *sdw1/denso* in the breeding process is of great importance in the development of modern varieties (Kuczynska et al., 2013).

The tremendous success of the ‘Green Revolution’ in the XX-th century was achieved due to the introduction of new grain varieties with shorter, durable stems resistant to lodging. This made it possible to change agricultural technologies, increase the dose of mineral fertilizers, that resulted in sharp grain crop yield increase. For wheat and rice, the Green Revolution genes were involved in gibberellic acid (GA) metabolism, affecting plant height (dwarf), lodging resistance, and, therefore, yield index (Teplyakova et al., 2017).

Earlier, it was also found out that alleles of the *sdw1/denso* locus have a significant impact on a number of agronomically valuable traits, in particular, plant height and earing period (Barua et al. 1993; Bezant et al. 1997; Laurie et al., 1995). It is known that the orthologous gene in rice semidwarf (*sd1*), localized on chromosome 1, encodes the enzyme gibberellic acid GK-20. The rice lines mutant with this gene are characterized by breach synthesis of gibberellic acids, in particular, by an increased accumulation of GK-53, a reduced content of GK-20 and bioactive GK-1.

The semidwarf varieties with the *Semidwarf 1 gene* (*sdw1/denso*) mutations are now widely spread among the sowings of short-stem barley fodder varieties in the western states of US, in Canada, Australia and among spring brewery varieties in Europe (Jia et al., 2009). Nowadays the varieties with the *sdw1/denso* gene are included in the genealogy of most modern barley varieties (Grausgruber et al. 2002; Dahleen et al. 2005).

The *sdw1/denso* gene has a pleiotropic effect, that is, it affects many traits, including the general adaptability and seed productivity of plants. It was established that the *sdw1/denso* gene affects not only plant height, but also grain quality and productivity (Jia et al., 2009). Therefore, it is necessary to identify such agrobiological traits, which depend on the allelic diversity of the *sdw1/denso* gene, as lodging degree, yield parameters, earing period, etc.

The purpose of the study was to compare the allelic polymorphism of the *sdw1/denso* gene with the results of a phenotypic assessment of economically important traits of winter barley in the field, thus establishing prospects for the use of functional (gene-specific) markers for marker-assisted breeding of highly productive barley varieties.

MATERIAL AND METHODS

Plant material. The objects of study were 96 barley varieties of local and foreign breeding, which were analyzed using the developed test systems of functional gene specific markers. This made it possible to estimate the efficiency of the developed molecular markers for marker-assisted breeding of high productive barley varieties in the Southern Federal District of the Russian Federation (SFD RF).

Collection samples were analyzed according to such main economically valuable traits of greatest interest as productivity, 1000-grain weight, grain protein percentage, lodging resistance and the length of the ‘sprouts-earing phase’ period.

1000-grain weight was determined according to GOST 10842-89 (2009). The grain protein percentage was determined according to GOST 10846-91 (2009). An assessment of lodging resistance was carried out according to the International Classifier of COMECON of Hordeum L. (1983) on a 5-point scale, where 1 is very low, 2 is low, 3 is medium, 4 is high, 5 is very high. The length of the period ‘sprouts-earing phase’ is a sum of the days from the period ‘full germination’ till the period ‘full earing phase’, including winter period (Table 1).

Table 1. List of the barley varieties and breeding line with characteristics of economically valuable traits

№	Name of the variety	Country of origin	Productivity, t/ha	1000-grain weight, g	Protein percentage, %	Resistance to lodging, point	Period ‘sprouts-earing’, days	*sdw1/denso*
1	Grand	Russia	6,5	39,1	12,4	5	224	R
2	Gorizont	Russia	5,2	38,3	12,6	4	223	R
3	Rostovsky 908	Russia	5,5	40,4	12,5	4	219	R
4	Donskoy 11	Russia	5,5	39,7	12,2	5	225	R
5	Rostovsky 55	Russia	6,4	40,6	12	5	223	R
6	Polet	Russia	6,3	39,4	11,7	5	225	R
7	Zhiguli	Russia	6,5	37,1	12,4	5	224	R
8	Larets	Russia	5,8	36,5	11,8	5	224	R
9	Parallelum 1920	Russia	5,7	40	11,9	4	222	R
10	Parallelum 1921	Russia	5,6	38,7	11,5	4	222	R
11	Parallelum 1922	Russia	6,1	40,5	11,5	4	221	R
12	Parallelum 1923	Russia	6,8	39,6	11,4	5	219	R
13	Parallelum 1924	Russia	6,6	40,5	11,3	5	221	R
14	Pallidum 1926	Russia	6,2	39,5	11,8	4	220	R
15	Mikhaylo	Russia	6,3	39,5	11,8	5	221	R
16	Fakir	Russia	6,8	43,6	12	5	222	NA
17	Premier	Russia	5,6	52,5	13	5	220	NA
18	Vavilon	Russia	5,7	41,6	12,4	5	221	R
19	Skorokhod	Russia	5,9	41,8	12,6	5	216	R
20	Bastion	Russia	5,4	37,9	12,5	4	222	R
21	Kordon	Russia	6,3	40	12,7	5	220	R
22	Sekret	Russia	6,5	41,6	12,8	5	216	R
23	Kozyr	Russia	5,9	39,7	12,4	5	219	R
24	Radikal	Russia	5,3	36,4	12,4	4	219	R
25	Avans	Russia	6,1	37,9	12,5	5	220	R
26	Kubanets	Russia	6,5	40,5	13	5	219	R
27	Platon	Russia	7,3	43,1	12,4	5	220	R
28	Khutorok	Russia	7,3	41,4	11,9	5	221	R
29	Romans	Russia	7,7	39,4	11,6	5	218	R
30	Samson	Russia	6,9	43,5	12	5	223	R
31	Gordey	Russia	7,6	41	12,4	5	219	R

32	Kondrat	Russia	7,1	41,2	12,4	5	221	R
33	Dobrynya 3	Russia	6,4	39,5	12,5	5	221	R
34	Lazar	Russia	7,1	40,8	11,7	5	221	R
35	Rubezh	Russia	8	45,2	12,5	5	220	R
36	Aborigen	Russia	6,6	40,7	11,7	4	220	R
37	Trudivnik	Russia	6,6	42,5	11,8	4	219	R
38	Selena Star	Russia	6	39,5	12,2	4	220	R
39	Metelitsa	Russia	5,9	39,9	11,7	4	220	DR
40	Sinelnikovsky 56	Russia	5,1	31,8	13,2	4	225	R
41	Bezosty	Russia	5,1	35,1	13,4	5	224	R
42	Zolak	Czech R.	5	42,1	12,5	4	220	R
43	Okal	Czech R.	6,6	43,4	12,5	5	224	R
44	Esterel	France	6,1	42,4	11,8	5	220	DR
45	Azurel	France	6,5	42,7	11,6	4	220	DR
46	Capten	France	6,5	43,5	11,9	5	220	DR
47	Broinskayli	France	6,3	55,4	12,7	5	223	D
48	Nectaria	France	5,6	54,6	12,9	5	220	DR
49	Vanessa	France	5,9	51,9	12,5	4	223	D
50	6577CH	France	4,7	47,9	13	5	221	DR
51	18513EH11	France	4,7	52,8	13,1	5	221	DR
52	Docile	France	5,7	42,3	12,7	5	221	DR
53	Caprice	France	4,9	39,7	12,4	4	220	R
54	Marissa	France	7,4	46,7	12	5	222	R
55	HVW 1427	Germany	6,2	39,7	12,3	4	219	R
56	Cotanici	Germany	5,5	40,6	12,5	4	220	R
57	Trasco	Germany	5,9	41,3	12,3	4	222	DR
58	Cita	Germany	6	41,7	11,6	5	221	R
59	Rocca	Germany	6,3	44,8	11,1	4	220	DR
60	Ushi	Germany	6	43,1	11,5	4	221	R
61	Tokyo	Germany	6,5	43,9	11,6	5	221	DR
62	Blanka	Germany	6,2	42,4	12,2	5	220	R
63	Nixe	Germany	6,3	42,7	12,2	5	220	R
64	Punch	Germany	5	47,9	13,3	5	221	R
65	Tiffany	Germany	6,4	45	12,4	5	222	DR
66	Arkona	Germany	6,4	44,9	12,6	5	223	R
67	Cornelia	Germany	6,3	43,3	12,4	5	222	R
68	Tsindarella	Germany	6,5	44,8	12,1	5	222	R
69	Willis	US	5,5	33,5	12	4	223	R
70	Callao	US	5,7	38,6	13,1	5	217	R
71	Parallelum 1904	Russia	6,6	35,4	11,8	5	225	R
72	Parallelum 1883	Russia	6,8	39,8	11,7	4	220	DR
73	Parallelum 1767	Russia	7,2	41,9	11,3	5	221	R
74	Parallelum 1911	Russia	7,1	39,7	11,9	5	220	R
75	Parallelum 1912	Russia	6,9	39,4	11,9	4	218	R
76	Parallelum 1914	Russia	6,7	40,5	11,8	5	224	R
77	Pallidum 1916	Russia	7,2	45,9	11,6	4	221	R
78	Parallelum 1917	Russia	7,1	40,4	11,7	5	216	R
79	Parallelum 1930	Russia	7,1	38,6	11,8	5	220	R
80	Vivat	Russia	7	40,3	12,5	5	223	R
81	Randevu	Russia	5,4	56,7	12,8	5	219	DR

82	Parallelum 1949	Russia	6,2	38,2	11,9	5	219	R
83	Nutans 1896	Russia	6,2	55,2	12,5	5	219	NA
84	Nutans 1895	Russia	5,9	52,8	12,1	5	220	NA
85	Parallelum 1906	Russia	6,7	40,2	11,7	5	224	R
86	Parallelum 1893	Russia	6,4	36,5	12,2	5	225	R
87	Erema	Russia	6,6	37,5	11,8	4	219	
88	Tigr	Russia	6	42,1	11,6	4	217	R
89	Parallelum 1950	Russia	6,3	40	11,9	4	218	R
90	Pallidum 1925	Russia	6,6	41	11,6	4	217	R
91	Timofey	Russia	6,8	39,5	11,7	5	223	R
92	Bezosty 1954	Russia	5	39,6	11,6	4	219	R
93	Bezosty 1955	Russia	5,4	40,2	12,3	5	219	R
94	Larets	Russia	6,3	36,7	13,1	5	225	DR
95	Polet	Russia	6,4	40	12,2	5	223	R
96	Zhiguli	Russia	6,1	36	12,3	3	223	DR

Molecular analysis. For molecular genetic screening of the studied samples, the winter barley seeds were disinfected for 2 minutes in 5% KMnO4 solution, then thoroughly were washed with distilled water and germinated on moist filter paper in Petri dishes for 4-5 days at 25 ° C. The genomic DNA was isolated from the barley leaves by the standard method using the STAB-buffer (Saghai-Maroof et al., 1984). The quality and quantity of the isolated DNA was verified by a Shimadzu UV mini-1240 spectrophotometer. PCR was performed in a GeneAmp PCR system 9700 amplifier. The PCR products were visualized by electrophoresis in a 1.3% agarose gel with a 1% solution of ethidium bromide in 0.5 × TBE buffer (90V by 14.5 cm). The composition of the PCR reaction mixture with primers and data on the molecular marker developed for the *sdw1/denso* gene are given in Tables 2 and 3.

Table 2. Composition of the reaction mixture PCR with primers for the *sdw1/denso* gene alleles. The used primers are Hv20ox2F_1: GCTGATTAACTGGGACACATA, Hv20ox2R_1: GGTGCTAATGTCGCTATGTC

Composition of the reaction mixture	Volume of a component	Initial concentration	Final concentration
DNA	2 microliter	~ 60 ng/ microliter	~ 6 ng/ microliter
10xbuffer for Taq polymerase, pH .,6, 25mM Mg2+	2 microliter	10x	1x
dNTPs (10мМ)	1 microliter	10 mmole	500 mmole
Hv20ox2F_1	0.5 microliter	10 mmole	0.25 mmole
Hv20ox2R_1:	0.5 microliter	10 mmole	0.25 mmole
Taq polymerase	0.5 microliter	5 units/microliter	0.125 units/microliter
H_2O	14 microliter		
Total volume	20 microliter		

Table 3. The tested functional gene-specific molecular marker developed for the *sdw1/denso* gene, which determines the variability of economically valuable traits of barley

The tested allele of the gene	The sequence of allele-specific primers used in the analysis	Testing SNP	Tm Primers' anneal	Expected dimension of a DNA-	The author

			ing (t°)	fragment , pn	
Denso	Hv20ox2F_1: GCTGATTAACTGGGACACATA Hv20ox2R_1: GGTGCTAATGTCGCTATGTC	G/A (A)	60	194	Jia et al., 2009
denso	Hv20ox2F_1: GCTGATTAACTGGGACACATA Hv20ox2R_1: GGTGCTAATGTCGCTATGTC	G/A (G)	60	194/ *Hae III* = 107/87	Jia et al., 2009

The PCR product obtained with primers for *the sdw1/denso* gene was treated with the restriction endonuclease *HaeIII*. The restriction mixture was incubated at 37°C for 3 hours, then it was visualized by agarose gel electrophoresis.

Statistical analysis of phenotypic data. The efficiency of allele-specific markers for the rapid diagnosis of the parameters of the vegetation period, the protein percentage in grain, and the productivity indicators were estimated statistically by comparing the quantitative indicators of the studied trait in the varieties with alternative alleles of the tested gene. There was used the non-parametric Mann–Whitney U test to estimate statistically reliable (unreliable) differences in the field indicators between the varieties with alternative alleles of the genes. Reliability of differences was confirmed at the 5% level of significance.

RESUTS AND DISCUSSION

To identify alleles at the *sdw1/denso* locus, a CAPS marker was used to detect SNP in the second gene intron. According to preliminary data, a deletion in the first gene exon is functional, however, for marking it is used the SNP closely linked to it. The results of molecular screening are shown in Figure 1.

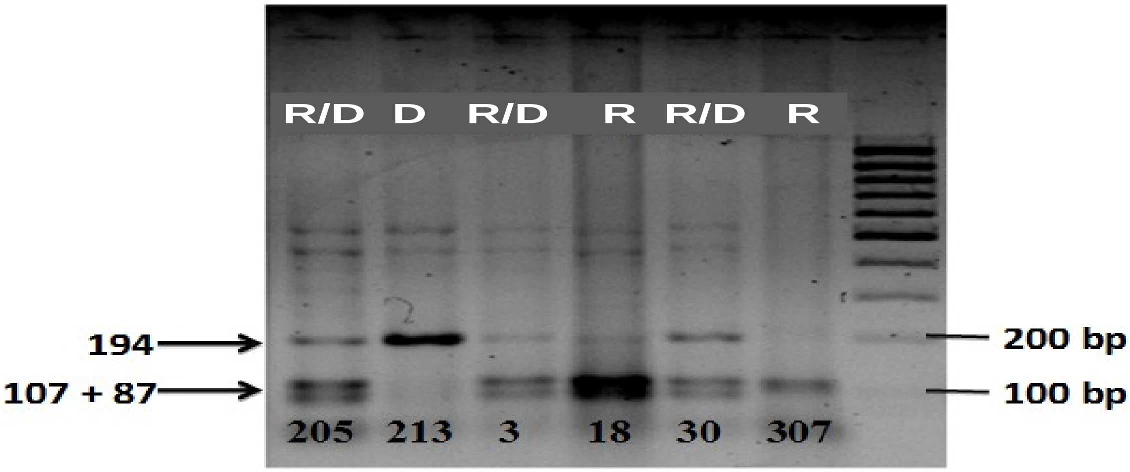

Figure 1. Identification of the dominant (194 pn) and recessive (107 + 87 pn) alleles of the *sdw1/denso* gene with the help of the CAPS marker in the winter barley varieties

The marker's testing allowed conducting a successful amplification for 91 varieties, where 76 varieties have a widespread recessive allele (as the variety 'Morex'), and 15 varieties have a dominant mutation (as the variety 'Barke').

This level of allelic polymorphism gave an opportunity to carry out a statistical analysis of the association between the *sdw1/denso* gene polymorphism and the variability of economically valuable traits of the barley varieties (Table 4). It has been determined that the *sdw1/denso* alleles reliably affect on such important yield element as 1000-grain weight ($p = 0.00002$).

Table 4. The effect of the dominant (D) and recessive allele (R) of the semi-dwarf *sdw1/denso* gene on the variability of five agrobiological traits in the winter barley varieties according to the Mann-Whitney U test

Trait	Sum of the ranks		p-level
	Allelic state		
	D	R	
Productivity, t/ha	517,50	3668,50	0,0650
1000-grain weight, g	1087,50	3098,50	**0,0000**
Protein percentage, %	815,50	3370,50	0,1795
Lodging resistance, mark	742,50	3443,50	0,5744
Period ‘sprouts-earing phase’, days	709,50	3476,50	0,8348

Among the barley varieties to be tested, 70% were bred in the FSBSI “Agricultural Research Center “Donskoy”, 15% were the varieties of European breeding and 2% were grown in the United States. In order to make sure that the identified association between *sdw1/denso* alleles and 1000-grain weight is not related to the origin of the variety, two-factor analysis of variance was performed, and its results are presented in Table 5 and Figure 2.

Table 5. The results of two-factor analysis of variance to identify the association of the effect of the *sdw1/denso* gene alleles and the variety origin on 1000-grain weight in winter varieties

Factors, affecting the variability of 1000-grain weight	p
Intercept	0,000000
Alleles *sdw1/denso*	**0,000013**
Variety origin	**0,023560**
Association of both factors (sdw1/denso*Origin)	0,698007

p – level of significance

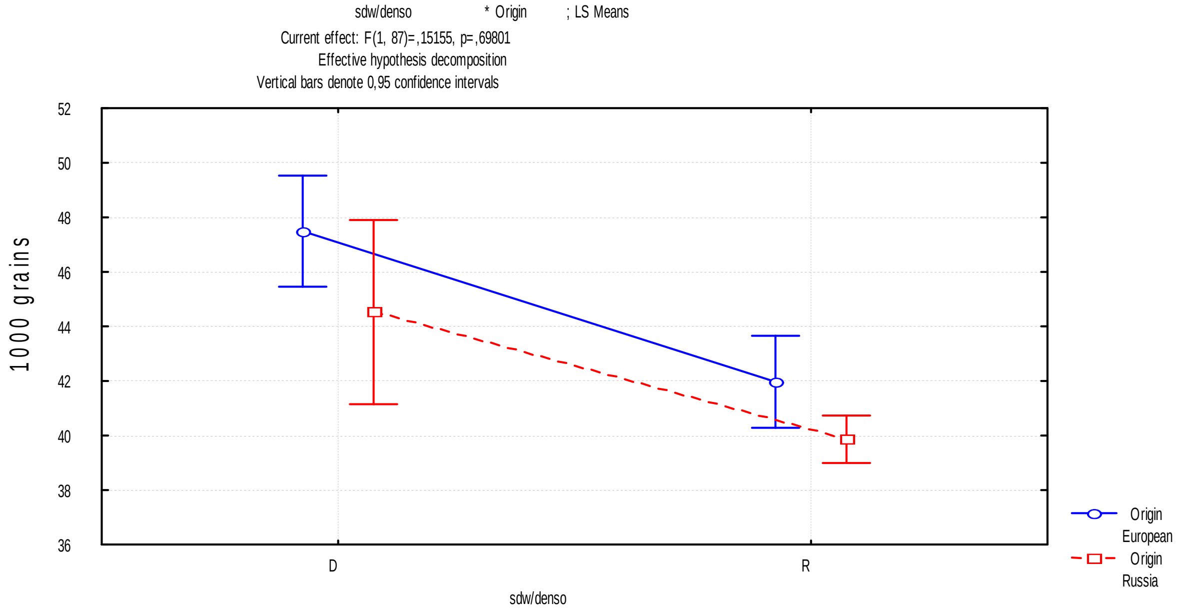

Figure 2. Comparison of the average 1000-grain weight (g) in winter barley varieties depending on the presence of the dominant (D) and recessive (R) allele of the *sdw1/denso* gene and the variety origin (Origin)

It has been established that *sdw1/denso* alleles, as well as the variety origin, affect this important yield indicator, but both factors are not connected with each other. Thus, the dominant (mutant) allele of the *sdw1/denso* gene, identified by the CAPS marker, can be recommended for selection of more productive genotypes.

The results of the conducted researches of the test systems of functional molecular markers over the candidate genes are summarized in Table 6.

Table 6. Map of researches of the test systems of functional molecular markers over the *sdw1/denso* gene

Technical criteria					Criterion of association with agronomic traits (p-value)				
Marked genes	Responsiveness	Reproductivity	Peculiarity	Polymorphism level	Productivity, t/ha	1000-grain weight, g	Protein percentage, %	Lodging resistance, mark	Period ‘sprouts-earing phase’, days
sdw1/ denso	95%	100%	100%	16%	0,07	**0,00**	0,18	0,57	0,83

It was established that the *sdw1/denso* gene alleles are reliably associated with ‘grain size (coarseness)’ as one of the main parameters of yield.

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