GROWTH AND VIABILITY OF COLEOPTILES UNDER OXYGEN DEFICIENCY IN *Oryza sativa* L. FROM THE COLLECTION OF THE FEDERAL RICE RESEARCH CENTER

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A b s t r a c t

The distinctive ability of rice seedlings lies in the ability to germinate under conditions of oxygen lack. At the germination stage, the first to develop is the coleoptile, a juvenile organ that protects the true leaf in cereals. The mechanisms of regulation of growth and development of this organ have not been studied to a large extent. Special attention may be paid to a coleoptile in seedlings capable of germinating under oxygen conditions. In the presented study, for the first time, the importance of the growth rate and viability of coleoptiles of rice seedlings during flooding in deter-mining survival and development was demonstrated. A total of 36 varieties and forms from the collection of the Federal Research Center for Rice, Krasnodar, were tested. Sprint and Kuban 3 were among the fastest growing varieties. Their coleoptiles reached 19-25 mm length both under normoxia and hypoxia. The slowest growing group included three Philippine varieties harbouring the *SUB1A* allele (HHZ11 Y6-Y2-SUB1, HHZ8 SAL 14 SUB1, HHZ9 DT12 SUB1), Chinese vari-ety Xiannui and domestic varieties Amethyst, Zhemchug, Natasha, Rapan and Yuzhnaya noch. This group of varieties was characterized by inhibition of the growth of coleoptiles by 2.5-3 times under submergence. In main a positive correlation was estimated between the coleoptile length in normoxia and hypoxia ($\rho = 0.70$, $p = 10^{-6}$), i.e. forms that actively grow in an aerobic environment also grow rapidly when flooded. Further detailed analysis of the growth of coleoptiles under hypoxic conditions showed that growth changes correspond to several patterns. The most common reaction to hypoxia in rice coleoptiles of the first group was growth suppression, but with the preservation of a small part of the plants that continue to grow. This group included all Philippine cultivars harbouring the *SUB1A* allele. In the second group, a significant proportion was plants which length under submergence ex-ceeded that at normoxia. Approximately half of the plants of the third group slowed growth arrest, while the other part continued to grow as in normoxia. The fourth group included the variety Sprint, which was the only one to have traits of avoidance strategy (LOES — low oxygen escape syndrome) associated with growth enhancement, although this enhancement was not intensive. The variety Yu-zhnaya noch had a unique growth pattern, the coleoptiles of which grew slowly both in normoxia and being submerged. In addition to growth, the viability of coleoptiles was analyzed in the work, which

was assessed using a tetrazolium test. Under hypoxic conditions, the viability of all tested forms significantly decreased. In the fastest growing varieties (Sprint, Kuban 3), the color intensity of tetrazolium salts was higher both in the control (6-fold) and in the experiment (2-fold), compared to slow growing forms (Amethyst, Yuzhnaya noch, Philippine *SUB1A* varieties). In general, growth rate correlates with metabolic rate and submergence tolerance. Obtained results illustrate the tolerance to oxygen deficiency of the genotypes from the collection of the Federal Research Center for Rice, and show that coleoptile elongation can be used as a criterion for assessing the tolerance of rice varieties to the lack of oxygen.

Rice (*Oryza sativa* L.) is the oldest agricultural crop, the cultivation of which began more than 5 thousand years BC. It forms the basis of nutrition for several billion people. In 2022, 525.6 million tons of rice grain was harvested, which is grown on more than 167 million hectares (http://www.fao.org/worldfoodsituation/csdb/ru/). Rice is cultivated quite intensively in Russia. The area under rice as of June 2022 in the Russian Federation amounted to 162.5 thousand hectares (https://sfera.fm/news/zernovye/minselkhoz-otmetil-snizhenie-ploshchadi-seva-zerna-v-rf-na-07-mln-ga-god-k-god). In Kuban, the main rice-growing region of the Russian Federation (92.3 thousand hectares), the rice harvest according to Rosstat, in 2022 amounted to 582.6 thousand tons (https://rosstat.gov.ru/compendium/document/13277). Understanding the mechanisms of rice plant adaptation to floods facilitates breeding varieties resistant to long-term flooding in view to reduce the pesticide load on rice paddies and to ecologize commercial rice farming.

A distinguishing feature of rice plants is their ability to germinate under oxygen deficiency (hypoxia) or complete absence (anoxia). Plants growing in wetland environments, including rice, use two strategies to adapt to oxygen deficiency. The first strategy is aimed at actively avoiding oxygen deficiency (low oxygen escape syndrome, LOES), the second is a passive rest strategy (low oxygen quiescence syndrome, LOQS) [1].

The LOES strategy provides rapid elongation of shoots, hyponastic bending and changes in leaf anatomy to improve the diffusion of gases, the formation of aerenchyma, and an increase in the number of superficial adventitious roots [2-6]. The formation of aerenchyma necessary for the intensification of gas exchange between plant organs and tissues is under regulation by phytohormones, primarily ethylene. It is considered as a key regulator of the plant hormonal status under the LOES strategy. Ethylene initiates a decrease in the abscisic acid concentration and promotes an increase in auxin and gibberellins [3, 5, 7, 8]. In rice, the *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*) genes of the *ERF-VII* (ethylene response factor) family regulate the LOES strategy. With an increase in their expression in internodes, both the content of gibberellins and sensitivity to them increases, which stimulates the activity of intercalary meristems [9].

An important adaptation to oxygen deficiency is the formation of a gas film on the hydrophobic surface of leaves and stems of flooded plants. It is known that rice plants retain a surface gas film even during long-term (4-5 days) flooding [10]. The film is responsible for the delivery of oxygen from the above-water surface and is important for underwater photosynthesis, which provides plants with energy [5, 6]. Accelerated growth also occurs due to the metabolization of sugars with the participation of protein kinases. For example, CIPK15 (calcineurin B-like protein interacting protein kinase15) initiates a SnRK1A-dependent cascade of anaerobic starch degradation [11]. In turn, SnRK1A (sucrose-nonfermenting1-related protein kinase 1A), interacting with the transcription factor MYBS1, stimulates the expression of the α-amylase which is especially important for the starch breakdown during seed germination.

Inhibition of growth and metabolism are the main criteria for an alternative

LOQS strategy. This strategy means metabolic adaptations that prevent energy starvation, cytoplasmic acidification, and the toxicity of anaerobic metabolites. The energy generated during starch metabolism, the glycolysis and fermentation is primarily spent on transport processes, protecting cells from reactive oxygen species (ROS), and preventing protein denaturation [5, 11, 12]. In rice, ethylene is the main regulator of LOQS as in the avoidance strategy. The protein encoded by the *SUB1A* gene of the group of ethylene-sensitive transcription factors (ERF-VII) inhibits ethylene synthesis, leading to a decrease for gibberellins. This stimulates the accumulation of brassinosteroids, the growth regulators that promote the breakdown of biologically active gibberellins and the accumulation of the SLENDER RICE1 (SLR1) protein which suppresses the transduction of the gibberellin signal [13]. In addition, SUB1A inhibits sugar metabolism, reducing the expression of amylase and sucrose synthase genes [14]. When implementing the LOQS strategy, plant growth is inhibited during flooding, and the saved resources are used to wait out the period of oxygen deficiency and subsequent regrowth after gas exchange is restored.

The coleoptile is a special juvenile organ of seedlings in cereals. It grows through the soil, keeping the true leaves intact. If light hits the coleoptile, it stops growing and a true leaf grows through it. The coleoptile has a limited period of development, when all its cells undergo an extensional growth stage. It is believed that longer coleoptiles in many cases have the ecological advantage, for example, they provide protection from high temperature and dense environments [15, 16]. The ability to elongate the coleoptile has proven beneficial for crops in unfavorable conditions, as deep seeding is important for growth under more favorable temperature and humidity conditions, reduces the risk of damage from attacks by mice or other animals, and reduces damage from pre-emergence herbicides [17-19].

A significant number of factors have been identified that regulate coleoptile elongation at the transcription and post-translation levels [20, 21]. Extensional growth has been well studied in coleoptiles of cereals, in particular oats, corn and canarygrass. Note that in rice, at the germination stage, the coleoptile is the first to develop from the grain. The coleoptiles of this plant complete their growth on days 6-9. Under flooding, the growth of coleoptiles in rice varieties adhering to the LOES strategy is significantly accelerated, while in LOQS forms, on the contrary, it is inhibited [22]. In this regard, it is advisable to use the length of the coleoptile as a criterion when choosing genotypes that contrast in their ability to grow, and therefore, in resistance.

Currently, the Federal Research Center for Rice (Krasnodar) stores 7.3 thousand rice samples in the Unique Scientific Installation — UNU Collection of Rice Genetic Resources. The rice gene pool in UNU is represented by varieties, accessions, mutants, dihaploids of *O. sativa* of *indica* and *japonica* subspecies and includes 82 varieties from 42 rice-growing countries. The conserved gene pool is extensively studied to identify sources and donors of valuable traits [23, 24]. However, testing collection samples for resistance to various adverse environmental factors (including oxygen deficiency), unfortunately, is far from complete. In our opinion, the ability of rice plants to change their adaptation strategy under flooding conditions during early ontogenesis can largely determine the final crop yield. In addition, it is not clear whether the growth and stability of a juvenile organ can be extrapolated to the stability and development of an adult plant.

The presented work demonstrates for the first time the importance of the growth rate and viability of coleoptiles of rice seedlings under flooding in determining the stability of plants and their development in subsequent ontogenesis.

The purpose of the work was to assess the growth rate and viability of coleoptiles under flooding conditions in rice varieties and accessions from the collection

of the Federal Research Center for Rice.

Materials and methods. Growing rice (*Oryza sativa* L.) and phenotyping plants for economically valuable traits was carried out in 2019-2022 in a collection nursery (irrigation system of the Federal Research Center for Rice, Krasnodar, Belozernoye village).

The seeds of 36 varieties and accessions from the collection of the Federal Research Center for Rice were used. These were 24 rice varieties of domestic breeding (Amethyst, Anait, Veles, Viola, Vita, Gamma, Zhemchug, Krasnoarmeysky 313, Kuban 3, Leader, Natasha, Novator, Olimp, Privolny 4, Rapan, Regul, Sonata, Sprint, Titan, Ussur, Fontan, Khazar, Sharm, Yuzhnaya noch), one variety from Uzbekistan (Devra), three varieties from China (Xiannui, Zhongyon, Zhongyon 207) and eight samples from the International Rice Research Institute (IRRI, Philippines) (AA WAB 56-125, HHZ11 Y6-Y2-SUB1, HHZ8 SAL 14 SUB1, HHZ9 DT12 SUB1, IR 50, IR14 L 110, Kirkpinar, PV-1 IRBLSH). Among the Philippine accessions, there were three ones carrying the *SUB1A* allele characteristic of the most resistant and slow-growing LOQS rice varieties.

As part of international cooperation, rice samples of world selection entered the collection of the research center from various unified variety testing nurseries (IRRI, Philippines) in which various cultivation methods were prcaticized, e.g., with shortened flooding (IRLON), from nurseries of cold-resistant rice (IRCTN) and artificially irrigated rice from temperate latitudes (IRTON), of deep-water, flood-tolerant rice (IRLYN-SUB), of irrigated lowland rice from green technology nursery (GAR-IRLL), and irrigated super rice from green technology nursery (GSR-Rell) (http://www.knowledgebank.irri.org/images/docs/rice-standard-evaluation-system.pdf).

The growth rate of seedlings was determined visually on a 9-point scale, where 1 point is low, 3-5 points is medium, 7-9 points is high. The varieties with a high growth rate (9 points) at the initial stage of germination were genotypes with a seedling length on day 7 of more than 2 cm; 7 points correspond to 1.5-2.0 cm length, 3-5 points to 1.0-1.5 cm, 1 point to less than 1 cm. As a result, rice varieties and forms were grouped for low, medium and high early growth rate. The development of plants under normoxia was assessed for the total height, the length of the panicle, the duration of the development period before flowering and the total growing season. The duration of growing season was assessed from the date of germination to flowering, and then until the grain is completely ripe. Elements of productivity were determined, including 1000-grain weight, and the overall productivity of the panicle. For biometric analysis, test sheaves of 10 plants were selected from the plots.

For a series of lab studies, the seeds were surface sterilized with a 50% sodium hypochlorite solution for 15 min, washed 10 times with sterile water and soaked in hot water (55 °C) for 1 hour. Next, 50 seeds of control samples were placed in an enamel tray on glass bridges, covered with gauze. A 4% Knop nutrient solution [25] was poured into the tray to the level of the glass bridges, covered with glass and germinated under normal air access. For hypoxia, the seeds were placed in 750 ml containers filled with the same solution to the very top (water column height of 12 cm) and hermetically sealed with a lid. In both treatments, plants were grown for 4 days at 29 °C in the dark. On day 4, the oxygen concentration in the hypoxic solution averaged 0.46 mg/l. It was measured using an Expert-009 dissolved oxygen analyzer (Econix-Expert, Russia). Dishes, gauze and solutions for working with plants were previously sterilized.

To measure the length of coleoptiles, the seedlings were placed in Petri dishes, scanned using an HP ScanJet G2710 (Hewlett-Packard, USA) and the

images were digitized in the ImageJ program (version 1.8.0_172) (https://im-agej.nih.gov/ij/download.html). All plants from 50 sown seeds were examined. After scanning, 5 coleoptiles from the control and test plants were used to determine viability by the tetrazolium salt reduction test. Coleoptiles were placed in test tubes with 5 ml of 2,3,5-triphenyltetrazolium chloride (8 g/l) in 0.1 M sodium phosphate buffer (pH 6.9). The solution with pieces of plant tissues was vacuum-infiltrated at –70 Pa for 15 min and allowed overnight in a thermostat at 29 °C.

The next day, triphenylformazan (a reduced form of triphenyltetrazolium colored red) was extracted from plant tissues. Coleoptiles, washed 3 times with distilled water, were placed into 2 ml microtubes, added with 1.5 ml of 96% ethyl alcohol and heated for 10 min at 85 °C in a TDB-120 solid-state thermostat (BioSan, Latvia). Optical density was measured at $\lambda = 485$ nm on a SPECTROstar Nano spectrophotometer (BMG LABTECH GmbH, Germany). Viability was expressed in units of absorbtion A_{485} per seedling. Experiments were carried out in three biological replicates.

Statistical analysis was performed in the R language environment [26]. The mean values of the trait (M) and standard errors of the means ($\pm$SEM) were calculted. Normality was tested using the Shapiro-Wilk test. Most samples showed a non-normal distribution of lengths, so the nonparametric Mann-Whitney-Wilcoxon test was used for comparison. A t-test was used to compare tetrazolium staining effects. For multiple comparisons, p-values were corrected by FDR (false discovery rate) method. Checking whether the seedling length in hypoxia and normoxia has the same distribution patters was carried out using the Kolmogorov-Smirnov test with dissimilarity (D) and p-value calculations. The kernel density estimate of the random variable was analyzed using the Epanechnikov function. To determine the correlation, the Spearman coefficient (ρ) was calculated.

Results. According to phenotyping data, the development of *O. sativa* plants from the collection of the Federal Research Center for Rice did not depend on the country and nursery of origin (Table 1). Growth rate was assessed at the initial stages of seedling development. It is during this period that plants have to overcome a 10-centimeter layer of water formed as a result of flooding rice paddies after sowing ("shortened flooding").

The varieties Yuzhnaya noch, Natasha, Veles and HHZ8 SAL 14 SUB1, classified as low group with low growth intensity, had limited stem height (see Table 1). The tall group included tall plants of the varieties Devzra, Kirkpinar and Kuban 3. Interestingly, the tall group also included varieties that reached only half the height of the tall ones, the IR 50, Zhongyon and Zhongyon 207. However, we note that this mainly concerned varieties of foreign origin that were selected according to other criteria. In addition, plant tolerance to flooding varies greatly under different temperature conditions [27].

That is, the growth rate and stability of varieties bred in the Philippines or China can change significantly when grown in the conditions of the Krasnodar Territory. In 2022, during the sowing period (first ten days of May), the average daily temperature did not exceed 16-17 °C (https://o-pogode.ru/prognoz-may-2022/krasnodar). Perhaps this was the reason for the lack of a clear correlation between the growth rate at the initial stage and the subsequent development of rice plants (see Table 1).

The slowest growing varieties were three Philippine varieties carrying the *SUB1A* allele, the Chinese variety Xiannui and domestic varieties Amethyst, Zhemchug, Natasha, Rapan and Yuzhnaya noch. The length of their coleoptiles was apprx. 10 mm in the control and apprx. 3 mm in hypoxia that means a 2.5-3.0-fold growth suppression.

1. Characteriszation of *Oryza sativa* L. varieties and accessions of different origins from the UNU Collection of rice genetic resources of the Federal Research Center for Rice (Krasnodar) **when grown in the Krasnodar Territory** ($n = 10$, $N = 3$, $M\pm SEM$, 2019-2022)

Variety, accession	Country of origin	Year of seed reproduction	Growth rate, seed color	Days		Plant height, cm	Panicle length, cm	Spikelet number per panicle	Panicle productivity, g	1000-grain weight, g
				before flowering	vegetation					
AA WAB 56-125	Philippines	2021	B, IRCTN	84	128±3	93.0±3.5	16.9±0.4	104±6	2.2±0.2	30.2±0.2
HHZ11 Y6-Y2- SUB1	Philippines	2018	H, GAR-IRLL, IRLYN-SUB	102	140±2	73.0±2.8	15.4±0.6	56±10	1.0±0.1	21.7±0.3
HHZ8 SAL 14 SUB1	Philippines	2018	H, IRLYN-SUB	113	146±2	71.0±2.6	22.1±1.1	92±8	1.2±0.2	22.7±0.3
HHZ9 DT12 SUB1	Philippines	2018	H, GSR-Rell	104	140±2	70.0±3.5	17.4±0.3	99±7	1.3±0.2	14.0±0.1
IR 50	Philippines	2020	B, IRTON	80	118±3	66.0±2.8	17.8±0.6	82±12	1.3±0.3	18.7±1.2
IR14 L 110	Philippines	2020	C, IRLON	95	134±1	76.0±3.8	19.6±0.7	115±17	1.6±0.3	22.3±0.3
Kirkpinar	Philippines	2019	B, IRLON	84	125±2	113.0±4.1	16.2±0.5	94±6	2.7±0.4	35.2±0.5
Xiannui	China	2021	B, IRCTN	75	112±4	88.0±4.6	21.4±0.5	155±12	2.7±0.4	26.3±0.5
Zhongyon	China	2019	B, IRCTN	70	110±4	68.0±4.5	20.2±1.0	203±21	4.3±0.3	27.9±0.3
Zhongyon 207	China	2020	B, IRCTN	92	130±2	71.0±2.2	19.3±0.5	125±24	2.7±0.5	22.1±0.1
Ametist	Russia	2019	H	86	120±2	94.0±4.3	13.8±0.5	98±7	2.8±0.5	30.6±0.5
Anait	Russia	2021	H	67	102±1	90.0±7.3	18.4±0.6	78±10	2.9±0.5	37.2±0.5
Veles	Russia	2021	H	88	125±3	73.0±3.7	16.2±0.2	189±21	3.8±0.5	26.4±0.2
Viiola	Russia	2021	C, violet-grained	82	118±3	97.0±5.0	14.2±0.4	67±5	1.7±0.3	23.5±0.3
Vita	Russia	2021	B, violet-grained	70	106±4	88.0±3.3	18.3±0.4	121±13	2.6±0.6	22.1±0.3
Gamma	Russia	2019	B	77	115±4	79.0±6.1	16.0±1.0	124±12	3.4±0.6	26.8±0.5
Devzra	Uzbekistan	2020	B	70	105±2	130.0±5.4	20.5±0.7	82±7	2.6±0.4	30.7±0.5
Zhemchug	Russia	2019	B	67	102±2	90.0±4.8	13.9±0.3	88±12	2.0±0.2	24.0±0.2
Krasnoarmeisky 313	Russia	2020	B	61	98±3	105.0±2.2	15.4±0.2	76±10	1.4±0.2	30.2±0.5
Kuban 3	Russia	2021	B	63	100±4	114.0±3.5	16.0±0.4	67±15	1.6±0.2	28.7±0.5
Lider	Russia	2021	B	78	116±5	101.0±5.1	16.3±0.7	127±12	2.9±0.5	26.2±0.3
Natasha	Russia	2019	H	77	118±2	74.0±8.2	18.5±0.5	91±7	1.7±0.2	25.6±0.7

Novator	Russia	2021	B	60	98±2	83.0±3.7	15.1±0.2	86±10	2.3±0.3	24.6±0.2
Olimp	Russia	2019	B	85	122±4	89.0±3.4	15.3±0.4	130±15	3.0±0.7	23.5±0.5
PV-1 IRBLSH	Philippines	2020	B, red-grained	100	140±1	101.0±4.5	19.1±0.4	117±8	2.6±0.8	24.2±0.2
Privolny 4	Russia	2021	H	77	115±3	92.0±6.2	15.8±0.6	120±22	3.6±0.6	24.8±0.3
Rapan	Russia	2021	C	78	116±2	85.0±2.4	15.3±0.6	121±10	3.2±0.3	26.3±0.3
Regul	Russia	2021	B	82	118±2	92.0±2.7	16.5±0.5	105±15	2.5±0.3	30.4 ±0.5
Sonata	Russia	2019	H	84	120±2	87.0±2.5	14.3±0.4	97±12	2.5±0.3	26.7±0.2
Sprint	Russia	2021	B	74	106±4	87.0±7.1	18.1±1.1	135±24	3.8±0.5	27.7±0.9
Titan	Russia	2021	B	74	115±5	86.0±4.3	14.6±0.8	104±6	3.1±0.3	35.0±0.4
Ussur	Russia, Primorye	2020	B	63	98±1	86.0±6.0	19.7±0.6	122±10	3.2±0.3	29.2±0.4
Fontan	Russia	2020	B	64	102±3	100.0±5.1	22.1±1.1	102±7	2.2±0.4	26.4±0.5
Khazar	Russia	2021	H	74	110±4	89.0±3.2	17.1±0.6	103±13	2.7±0.4	26.6±0.3
Sharm	Russia	2019	B	65	100±2	84.0±4.1	19.8±0.8	102±22	2.3±0.2	26.9±0.5
Yuzhnaya noch	Russia	2021	H, violet-grained	80	120±2	68.0±3.3	16.5±0.5	96±9	2.3±0.5	21.3±0.3

N o t e. H, C, B — varieties and variety forms with low, medium and high growth rates, respectively, at the early stage of ontogenesis. All varieties are white-grained, with the exception of those for which a different grain color is indicated. IRLON is shortened flooding, IRCTN is cold-resistant nursery, IRTON is artificially irrigated, temperate rice, GARIRLL is irrigated lowland, green technology nursery; GSR-Rell is irrigated, super-rice, green technology nursery; IRLYN-SUB is deep-water rice.

It should be noted that of the five varieties that have anthocyanin-colored grains, i.e., Viola, Vita, PV-1 IRBLSH, Natasha and Yuzhnaya noch, the varieties Natasha and Yuzhnaya noch were turned out to be slow-growing. The slowest growing variety was Yuzhnaya noch, in which coleoptiles grew to 4.0 mm in the control and up to 2.5 mm in the test. Thus, our study for the first time revealed a slowdown in the growth of coleoptiles with a lack of oxygen for the varieties examined. However, the intensity of suppression under flooding was consistent with the native growth rate of rice seedlings in control.
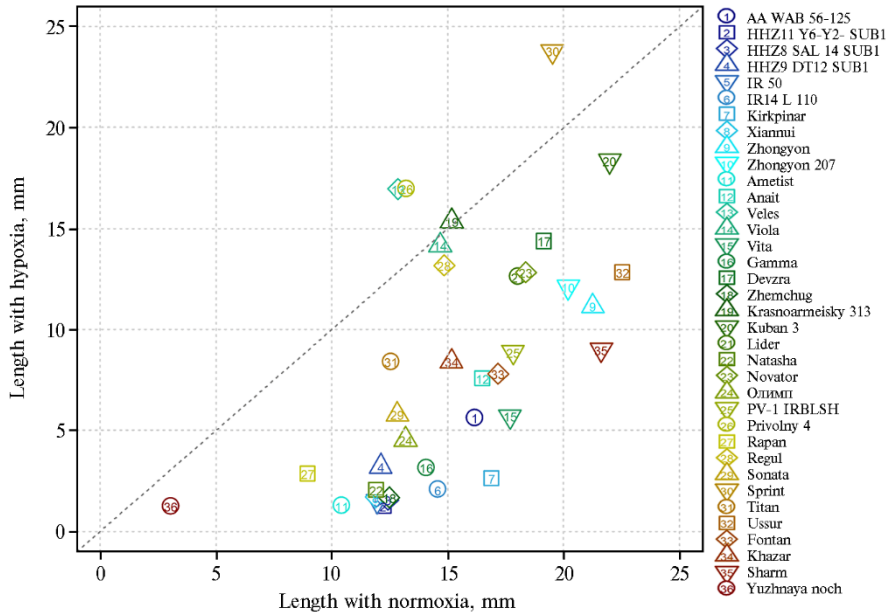

Fig. 1. Sample median of seedling coleoptile lengths in rice (*Oryza sativa* L.) varieties of different origins from the UNU Collection of rice genetic resources (the Federal Research Center for Rice, Krasnodar) **4 days after germination under normoxia and hypoxia** (lab test). The diagonal line corresponds to equal lengths for anoxia and hypoxia.

For most varieties and accessions, showed a decrease in the median (below the diagonal corresponding to equality) of coleoptile length during oxygen starvation (see Fig. 1), only in the varieties Viola and Krasnoarmeysky 313 the differences between normoxia and hypoxia were unreliable (Table 2). Under flooding, plant growth was most suppressed in HHZ11 Y6-Y2-SUB1, HHZ8 SAL 14 SUB1, IR14 L 110, IR 50, Xiannui, Amethyst, Zhemchug, Natasha and Yuzhnaya noch. Three varieties (Sprint, Veles and Privolny 4) showed a statistically significant ($p \leq 0.05$) increase in length under hypoxia (see Fig. 1, Table 2). It should also be noted that there is a positive correlation between the length of the coleoptile under normoxia and hypoxia ($\rho = 0.70$; $p = 10^{-6}$), that is, forms that actively grow in an aerobic environment also grew rapidly under flooding.

2. The difference reliability for the length of seedling coleoptiles in rice (*Oryza sativa* L.) varieties of different origins from the UNU Collection of rice genetic resources (the Federal Research Center for Rice, Krasnodar) **under normoxia, hypoxia and by the tetrazolium test** ($n = 5$, $N = 3$, $M \pm SEM$, lab test)

Variety, accession	Test values		Tetrazole test, rel. unts	
	Mann-Whitney-Wilcoxon	FDR	normoxia	hypoxia
AA WAB 56-125	4.32E–20	1.30E–19	0.077±0.007	0.008±0.001
HHZ11 Y6-Y2-SUB1	2.61E–08	3.91E–08	0.013±0.002	0.004±0.001
HHZ8 SAL 14 SUB1	8.05E–11	1.26E–10	0.027±0.005	0.005±0.001
HHZ9 DT12 SUB1	1.66E–13	3.41E–13	0.012±0.001	0.004±0.001

IR 50	5.52E–47	1.99E–45	0.050±0.012	0.006±0.001
IR14 L 110	1.15E–20	3.77E–20	0.131±0.017	0.008±0.0001
Kirkpinar	5.90E–31	1.06E–29	0.502±0.014	0.007±0.001
Xiannui	1.25E–17	2.99E–17	0.046±0.007	0.004±0.001
Zhongyon	3.46E–11	5.66E–11	0.269±0.013	0.005±0.001
Zhongyon 207	1.77E–14	3.99E–14	0.087±0.010	0.005±0.001
Ametist	4.94E–29	5.93E–28	0.057±0.009	0.006±0.001
Anait	6.64E–22	2.39E–21	0.083±0.011	0.006±0.001
Veles	2.69E–05	3.34E–05	0.090±0.005	0.008±0.001
Viiola	0.193686*	0.193686*	0.094±0.019	0.010±0.001
Vita	1.56E–18	4.02E–18	0.160±0.019	0.010±0.001
Gamma	5.98E–24	3.07E–23	0.221±0.018	0.010±0.001
Devzra	4.14E–07	5.74E–07	0.550±0.009	0.006±0.001
Zhemchug	5.05E–19	1.40E–18	0.275±0.025	0.005±0.001
Krasnoarmeisky 313	0.134287*	0.138124*	0.148±0.007	0.015±0.002
Kuban 3	0.000117	0.00014	0.172±0.009	0.017±0.001
Lider	4.98E–06	6.64E–06	0.232±0.010	0.010±0.001
Natasha	4.47E–27	3.22E–26	0.050±0.006	0.008±0.001
Novator	8.66E–08	1.25E–07	0.069±0.009	0.010±0.001
Olimp	2.94E–28	2.64E–27	0.201±0.043	0.006±0.001
PV-1 IRBLSH	6.87E–13	1.18E–12	0.028±0.003	0.010±0.001
Privolny 4	0.000828	0.000932	0.334±0.015	0.011±0.001
Rapan	3.53E–13	6.68E–13	0.020±0.004	0.004±0.001
Regul	0.001238	0.00135	0.160±0.006	0.010±0.001
Sonata	2.91E–22	1.31E–21	0.149±0.021	0.008±0.001
Sprint	1.58E–05	2.03E–05	0.129±0.024	0.011±0.001
Titan	0.004084	0.004324	0.092±0.013	0.014±0.001
Ussur	1.71E–13	3.41E–13	0.453±0.014	0.006±0.001
Fontan	2.34E–24	1.40E–23	0.107±0.022	0.010±0.001
Khazar	5.18E–13	9.33E–13	0.268±0.011	0.006±0.001
Sharm	5.55E–22	2.22E–21	0.234±0.005	0.011±0.001
Yuzhnaya noch	0.000305	0.000354	0.061±0.013	0.009±0.002

N o t e. FDR — false discovery rate. For all varieties, the normoxia and hypoxia variants in the tetrazolium test had statistically significant differences (*t*-test, p ≤ 0.05).
* Differences in conditions of normoxia and hypoxia are unreliable.

An adaptive strategy at the population level may involve changing distributions of trait values in response to changing environmental conditions. Histograms of probability density estimates for the rice seedling coleoptile lengths under various aeration conditions indicate that growth changes corresponded to several patterns.

The most common response (Fig. 2, pattern I) was a shift in the distribution to small length values (an increase in the asymmetry coefficient). Under hypoxia, histograms showed a high peak at small, close to zero values, in combination with an extended "tail" of values on the right. This indicates that the most common response to hypoxia in rice coleoptiles in this group was growth suppression, with a small portion of plants remaining continuing to grow. This strategy is apparently associated with the expectation of more favorable conditions and is close to the LOQS resting strategy. Moreover, it was this group that included all the Philippine varieties with the *SUB1A* allele which are the most prominent representatives of this adaptation strategy.

Pattern II (see Fig. 2) consisted of a decrease in the sharpness (kurtosis) of the probability distribution of length values under hypoxia. A significant proportion of this was made up of plants that were so large that there were none or almost none under normoxia. That is, in response to stress, the population distributed risks evenly across all possible options, which may allow at least some individuals to survive in an uncertain situation.

Pattern III was a combination of the previous two. Distributions during hypoxia became smoothed and shifted to the left. At the same time, most of them were concentrated in the area of small values, as well as in the area of values characteristic of normoxia. Approximately half of the plants slowed down, while

the other part continued to grow as under normoxia. Consequently, at the population level, the proportion of losses that depends on the choice of strategy can be recorded.
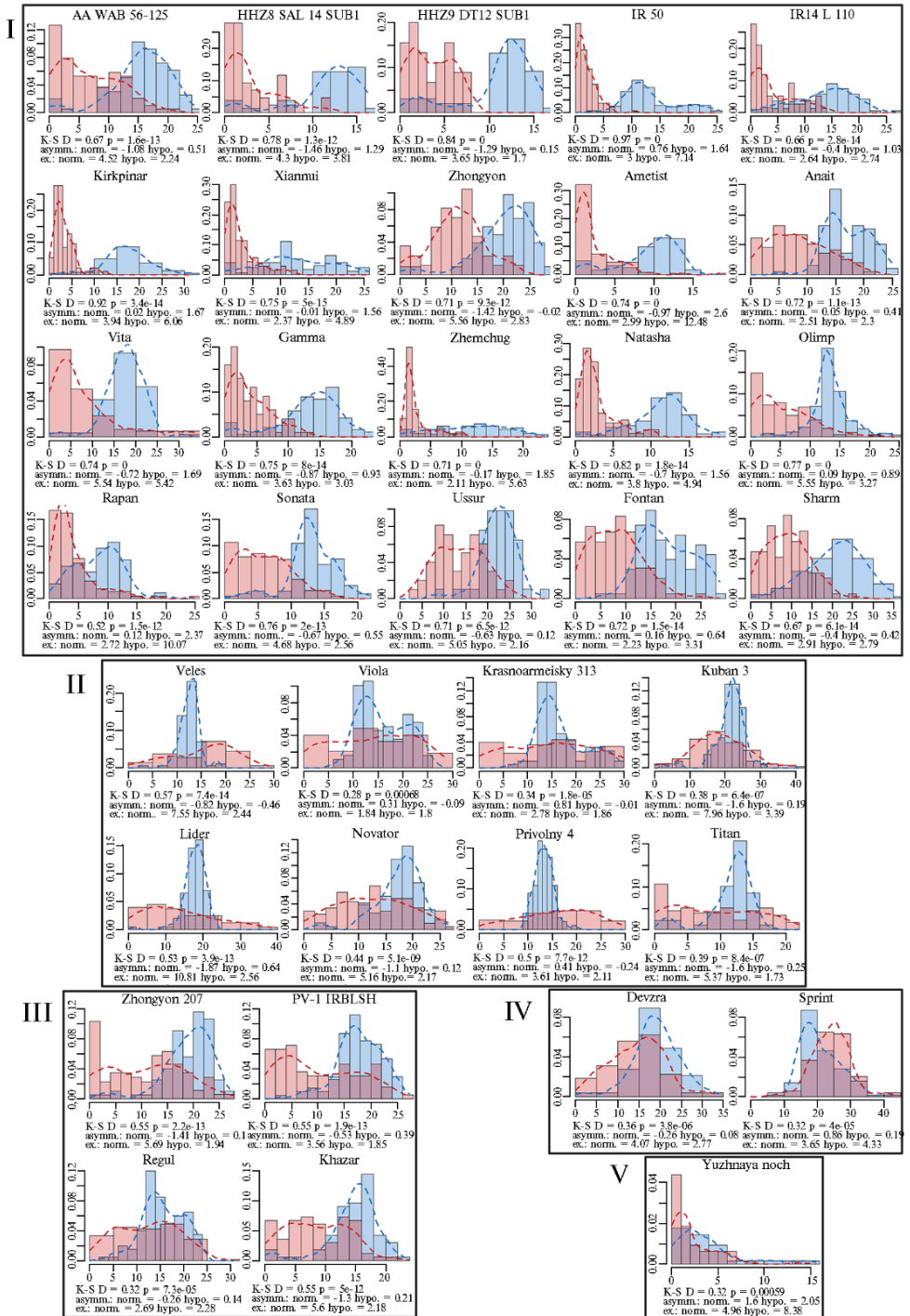

Fig. 2. Histograms of the seedling coleoptile length distribution in rice (*Oryza sativa* L.) varieties of different origins from the UNU Collection of rice genetic resources (the Federal Research Center for Rice, Krasnodar) **4 days after germination under normoxia** (blue) **and hypoxia** (red)**:** I-V — response patterns. Abscissa is length, mm, ordinate is probability density. Dashed lines are kernel density estimate with Epanechnikov function, K-S is Kolmogrov-Smirnov test, asymmetric. is asymmetry coefficient, ex. is kurtosis coefficient, norm. is normoxia, hypo. is hypoxia

Plants of the IV pattern showed the least changes in distribution. In the Devzra variety, there was a slight decrease in the spiciness of the distribution and a shift to the left which is similar to pattern III. In turn, we revealed a unique response in the Sprint variety, i.e., a clear shift to the right in the length distribution. That is, only this variety showed signs of LOES associated with a slight increase in growth. (see Fig. 1, 2). The Yuzhnaya noch also had a unique growth pattern (V pattern), the coleoptiles grew slowly both in the control and under flooding (see Fig. 2).

We can conclude that the growth response of coleoptiles that we identified corresponded to the onset of adaptation processes already at the first stages of ontogenesis during rice seedling development, and the patterns of changes in the growth response reflected the intensity of manifestation of one or another adaptation strategy.

It is well known that coleoptiles increase in length through cellular elongation growth [28]. Such growth, like any other, requires energy expenditure, but it is the cell's energy resources that are limited when there is a lack of oxygen. The diversity of growth response patterns is apparently due to changes occurring in the plant metabolism during hypoxia. Oxygen starvation leads to energy deficiency [3, 6]. Oxidative phosphorylation is inhibited, and the production of reactive oxygen species (ROS) is reduced. The only source of ATP is glycolysis, which turns into fermentation. Cytoplasmic acidosis and accumulation of toxic metabolic products, in particular acetaldehyde and ethanol, are triggered [1, 3, 6]. In addition, hypoxia inhibits the biosynthesis of protein and other polymers [6], induces the generation of reactive oxygen and nitrogen species [6, 29, 30] which cause oxidative damage to lipids and proteins [31, 32]. As discussed earlier, rice seedlings spend their meager energy reserves from starch metabolization depending on their tolerance strategy, plants with an avoidance strategy (LOES) spend on stimulating growth, and those with a growth inhibition strategy (LOQS) on maintaining cellular structures [2-6, 11, 12].
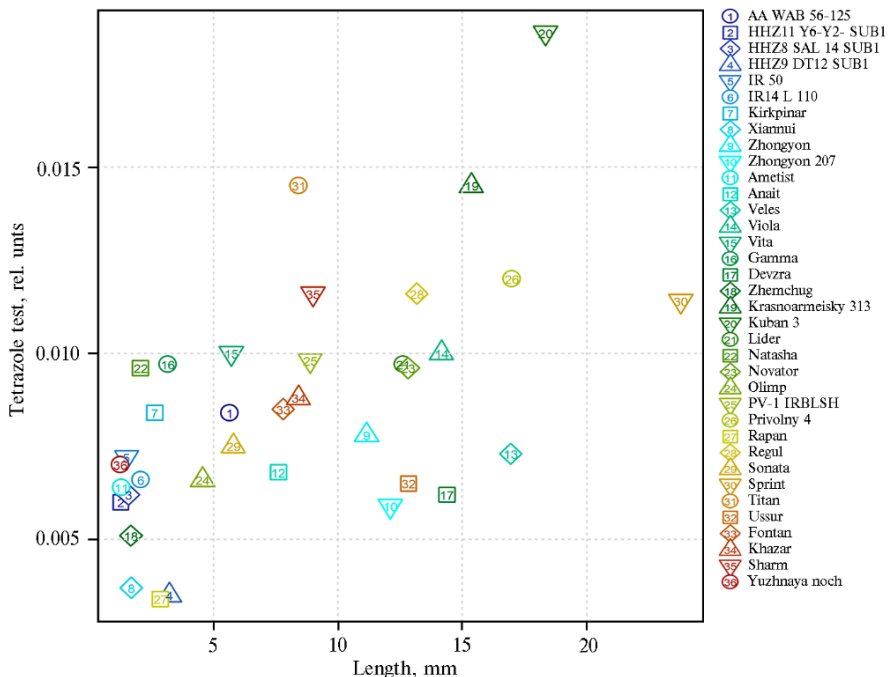

Fig. 3. Relationship between sample median of seedling coleoptile lengths in rice (*Oryza sativa* L.)

varieties of different origins from the UNU Collection of rice genetic resources (the Federal Research Center for Rice, Krasnodar) **4 days after germination under hypoxia with the results of the tetrazolium test.**

Thereof, we next focused on assessing the viability of coleoptiles in the tetrazolium test which assesses the metabolic rate. It is known that the more intense the color that develops in the test with tetrazolium salts, the higher the viability. In all tested forms, viability according to the tetrazolium test was significantly reduced under hypoxic conditions compared to the control (t-test, p ≤ 0.05, see Table 2). However, in slow-growing forms this decrease was not as strong as in fast-growing forms, which may be due to both the metabolic rate and the chosen adaptation strategy. The tetrazolium test characterizes the metabolic activity of tissues. In fast-growing forms, metabolism is intense, and under flooding, its inhibition was significant (by 10-20 times). In slow-growing forms, it is already slowed down, and its decrease during hypoxia was not so apparent, but in all cases statistically significant (by 7 times). Moreover, a positive relationship is shown between the intensity of tetrazolium staining and the length of the coleoptile ($\rho = 0.69$; $p = 10\ 5$), especially under hypoxia (Fig. 3). In the fastest-growing varieties (Sprint, Kuban 3, Krasnoarmeysky 313), the intensity of tetrazolium staining was higher both in the control (by 6 times) and in the test (by 2 times) compared to slow-growing forms (Amethyst, Rapan, Yuzhnaya noch, Xiannui, Philippine *SUB1A* varieties).

Variation in tetrazolium staining intensity has previously been used to test the viability of wheat and rice seedlings that differ in their tolerance to oxygen deficiency [33, 34]. It is shown that the test is applicable to assess the stability of the aboveground part. Rice shoots are less damaged under oxygen starvation.

Thus, the tested rice accessions from the collection of the Federal Research Center for Rice differed significantly in growth rate. In field trials, we identified three groups of varieties with high, medium and low growth rates at the germination stage. It was during this period that the developing seedlings were flooded. However, the growth intensity at the early ontogenesis did not correlate with the height of the adult plant, which can be explained by the lack of standardization of the germination stage (7 days), as well as the imposition of an additional stress factor, e.g., the low temperature (14 °C). The lab analysis of the coleoptile growth on day 4 showed that the fastest growing varieties were Sprint and Kuban 3, the length of their coleoptiles reached 19-25 mm both in the control and under flooding. Note that the domestic variety Kuban 3 reached its maximum height in field studies. Kuban 3 was assigned to pattern II type when analyzing the distribution of coleoptile lengths in seedlings of different varieties. The Sprint variety was assigned to pattern IV. The slowest growing domestic varieties were Amethyst, Zhemchug, Natasha, Rapan and Yuzhnaya noch. In the control, their coleoptile reached 10 mm, and in hypoxia apprx. 3 mm. All these varieties were assigned to pattern I togather with the Philippine varieties carrying the *SUB1A* allele and exhibiting a growth inhibition strategy (LOQS). Thus, our results indicate for the first time the variability of adaptation mechanisms which are reflected in growth rates already at the early stages of ontogenesis. Staining of coleoptile tissues with tetrazolium salts indicates inhibition of metabolism due to lack of oxygen. In fast-growing varieties, e.g., Kuban 3 and Sprint, the staining was 6 times more intense than in the slow-growing Amethyst, Rapan, Southern Night, Xiannui, and Philippine *SUB1A* varieties. Our data allow us to conclude that the growth rate of coleoptiles correlates with the intensity of metabolism and resistance to flooding. Unfortunately, the mechanisms of coleoptile growth under oxygen deficiency have not yet been fully established. Continued research is required to

understand which resistance strategies the tested varieties belong to and what factors determine their possible relationship. However, the presented results have already characterized the resistance to oxygen deficiency of the genotypes stored in the collection of the Federal Research Center for Rice.

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