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GROWTH OF BUCKWHEAT (*Fagopyrum esculentum* Moench) SEEDLINGS AND THE ACCUMULATION OF PRIMARY AND SECONDARY METABOLITES UNDER VARIOUS MINERAL NUTRITION CONDITIONS

E.V. TSYPURSKAYA[1], V.V. KAZANTSEVA[1], A.N. FESENKO[2], N.V. ZAGOSKINA[1]

[1]*Timiryazev Institute of Plant Physiology RAS*, 35, ul. Botanicheskaya, Moscow, 127276 Russia e-mail elena-pr22@mail.ru (corresponding author ✉), k.v.-90@mail.ru, nzagoskina@mail.ru;
[2]*All-Russian Research Institute of Legumes and Groat Grops*, 10/1, ul. Molodezhnaya, pos. Streletskoe, Orel, 302502 Russia, e-mail fesenco.a.n@rambler.ru
ORCID:
Tsypurskaya E.V. orcid.org/0000-0003-4897-531X
Kazantseva V.V. orcid.org/0000-0002-7031-8634
Fesenko A.N. orcid.org/0000-0002-7658-3471
Zagoskina N.V. orcid.org/0000-0002-1457-9450
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A b s t r a c t

Buckwheat (*Fagopyrum esculentum* Moench) is an important agricultural crop; Russia, China and Ukraine are the world leaders of its production. In addition to the unique nutritional characteristics, it is characterized by the formation of various phenolic compounds including rutin widely used in medicine. The study of the various metabolites formation at the initial growth stages as well as those under the different conditions of mineral nutrition is important for estimation of plant potential productivity and adaptation to environmental conditions. In this paper, we showed the regulatory effect of macro- and microelements on the growth and accumulation of primary and secondary metabolites in buckwheat plants. For the first time, the formation of primary and secondary metabolites in the aerial parts of a new and promising Russian buckwheat variety Dasha (approved by the State Register of the Russian Federation in 2018) has been characterized. The aim of this work was to study the initial stages of *F. esculentum* ontogenesis, including the assessment of the morphophysiological characteristics of seedlings under various mineral nutrition conditions, as well as the accumulation of photosynthetic pigments, sugars, and phenolic compounds in cotyledon leaves. Studies were conducted using two varieties of this culture included in the State Register of the Russian Federation in 2004 and 2018 (Devyatka and Dasha, respectively). Plant cultivation was carried out by a roll method in water (control) and Hoagland-Arnon nutrient medium (sample) at 24 °C and 16-hour illumination in laboratory conditions. In the seedlings, the height of the hypocotyls, the length of the roots, and the mass of cotyledon leaves was determined. The water content of the plant material was analyzed after it was dried to constant weight at 70 °C. The spectrophotometric method was used to determine the amount of chlorophylls a and b (λ = 665 nm and λ = 649 nm), carotenoids (λ = 440 nm), sugars (λ = 490 nm), the total amount of soluble phenolic compounds (λ = 725 nm), flavonoids (λ = 415 nm) and phenylpropanoids (λ = 330 nm) in ethanol extracts from cotyledon leaves of seedlings of different ages. The cultivation of buckwheat on Hoagland-Arnon nutrient medium contributed to faster growth of aboveground organs compared to control; in contrast, the growth of underground organs was the same in both cases. In most cases, in the experimental samples, the differences in the accumulation of photosynthetic pigments (chlorophyll a and b, carotenoids) and soluble sugars in the cotyledons of two buckwheat varieties were revealed to be higher than in control. As for the accumulation of phenolic compounds, it was not obviously dependent on the level of mineral nutrition. As an exception, in cotyledons of seedlings cultivated on a nutrient medium, the content of phenylpropanoids changed to a greater extent compared to control and reached high values at the end of the investigation period. It should also be noted that on a nutrient medium at the late ontogenesis stages cotyledons of Dasha seedlings significantly accumulate pigments, sugars and phenolic compounds in comparison with Devyatka. Thus, the obtained data indicate that the amount of mineral elements is important for the initial stages of *F. esculentum* ontogenesis. Faster growth of seedlings and the accumulation of primary and secondary metabolites in their leaves is characteristic of the experimental samples, compared to the control. Therefore, in plant cultivation, the different levels of mineral nutrition make it possible to regulate the plant growth and development, as well as the accumulation of various metabolites.

Keywords: *Fagopyrum esculentum* Moench, buckwheat, seedlings, ontogenesis, mineral nutrition, pigments, sugars, phenolic compounds, phenylpropanoids, flavonoids

Forming and development of seedlings is an important stage of ontogenetic development of plants that depends on endogenous stockpile of seed metabolites and their conversions, as well as impact of exogenous environmental factors, including soil humidity, temperature, light, mineral nutrition [1, 2]. For this period, transition from heterotrophic to autotrophic nutrition type, changes in structural arrangement of cells and tissues, energy processes (respiration, photosynthesis), state of hormonal, antioxidative and other systems are typical [3-5].

Some of important regulators of plant viability are phenolic compounds, one of the most widespread secondary metabolites present in all cells and tissues [6, 7]. Their content depends on plant species, ontogenesis phase, conditions of growing and mineral nutrition [8-10]. Functionality of phenolic compounds is extremely diverse and connected with the processes of photosynthesis, respiration, growth and development of plants, as well as stress-resistance [6, 11, 12].

Buckwheat (*Fagopyrum esculentum* Moench) is an important agricultural crop; it is cultivated in many countries of the world and is successfully used in various industries. Buckwheat is characterized by considerable accumulation of phenolic compounds, including rutin, a substance with high capillary-restorative effect [13, 14]. The largest concentration of these secondary metabolites was found in aboveground plant organs, especially in leaves and flowers [15, 16]. There were reports of formation of phenolic compounds in seedlings, where their amount was less and the composition was less diverse as compared to adult plants [17, 18]. Since phenolic compounds possess high biological and antioxidative effects, including as potential functional nutrition components [13], studying their accumulation during the initial plant ontogenesis phases is of practical interest.

In this paper, having compared a number of morphological, physiological and biochemical indicators of two buckwheat varieties (Devyatka and Dasha) under different mineral nutrition, we have discovered the regulatory effect of macro- and microelements on the early ontogenesis processes and accumulation of primary and secondary metabolites in aboveground organs at certain variety specificity of plant responses. For the new promising Dasha variety that had been entered in the State Register of Selection Achievements Authorized for Use in the Russian Federation in 2018, these processes are characterized for the first time.

Our goal was to evaluate the features of initial ontogenesis stages, morphophysiological characteristics and accumulation in cotyledon leaves of photosynthetic pigments, sugars, and phenolic compounds in buckwheat seedlings depending on the provision with mineral nutrition elements.

Techniques. Devyatka and Dasha variety buckwheat studied [19, 20] were obtained in Russian National Research Institute of Leguminous Crops and were entered in State Register of Selection Achievements Authorized for Use in the Russian Federation in 2004 and 2018, respectively.

The seedlings were grown by a roll method [10]. The seeds were placed in Petri dishes on a watered filter paper (control) and Hoagland-Arnon nutrient medium (sample) [21]. After curing in the dark for 24 hours, they were moved to the filter paper rolls (15 per roll) that were placed in the plastic tumblers (7 rolls per tumbler) containing water or nutritious medium, and were grown in phytotron chamber of the Institute of Plant Physiology of the Russian Academy of Sciences at 24 °C and 16-hour photoperiod (5 000 lux). The seedlings that were at the same phase of ontogenetic development were taken for study: for those grown on water, it were the 11th, 14th and 18th days of growth, and for the seedlings grown on nutrient medium the 6th, 11th and 14th day (respective phases 1st, 2nd and 3rd). The criteria were the form and size of cotyledon leaves that were used for biochemical study.

Morphophysiological parameters of seedlings, i.e. height of aboveground part and root length, as well as cotyledon leaves weight, were assessed. Tissue water content was determined after dehumidification of vegetation material at 70 °C in a thermostat to constant weight [10).

In order to extract the pigments, seedling leaves were homogenized in a 96% ethanol in the dark. Homogenate was centrifuged (CM-50, ELMI Ltd., Latvia) for 5 minutes at 13 000 rpm. Spectrophotometric method (SF-46, LOMO, Russia) was used to define *a* and *b* chlorophylls (λ = 665 nm and λ = 649 nm, respectively) and carotenoids (λ = 440 nm) in the supernatant liquid. They were measured by standard method [22].

Sugars were extracted by 96% ethanol extraction [23]. In the supernatant liquid resulting from centrifuging homogenate (2 minutes, 16 000 rpm), total sugar content was defined by spectrophotometric method through reaction with phenol and sulfuric acid (absorption at λ = 490 nm) [24]. Sucrose was used to construct calibration curve.

In order to extract phenolic compounds, vegetation material was homogenized in 96% ethanol and cured at 45 °C for 45 minutes [10, 24]. Homogenate was then centrifuged (2 minutes, 16 000 rpm). Supernatant liquid was used to identify different classes of phenolic compounds by spectrophotometric method. Content of the total phenolic compounds was evaluated through use Folin-Denis reagent (λ = 725 nm), flavonoid content – with 1% water solution of aluminum chloride ((λ = 430 nm). Phenylpropanoid amount was measured by direct spectrophotometry at λ = 330 nm. Rutin calibration curve was used for measuring total phenolic compounds and flavonoids, caffeic acid calibration curve — for phenylpropanoids. Experiments were arranged in triplicate biological and duplicate analytical replications.

Analysis of variance (ANOVA) was carried out with SigmaPlot 12.3 (http://www.sigmaplot.co.uk) and Microsoft Excel software. Tables and charts contain arithmetic means (*M*) and standard errors of mean (±SEM). Superscripts represent the significance of differences of mean values determined by Tukey test at $p \leq 0.05$.

Results. An important indicators of plant growth and development are their morphophysiological properties that depend on the ontogenesis phase, species and variety peculiarities and impact of external factors, including mineral nutrition [2, 4, 8].

Devyatka and Dasha buckwheat varieties are mid-season, high-yield and lodge-resistant [25, 26]. During the breeding of Dasha variety, the selection criteria were high grain content and photosynthetic activity [20]. This variety is characterized by a more pronounced resistance to drought, Ascochyta stem blight and mildew as compared to Devyatka variety [26]. Hence it can be assumed that there are certain differences in morphophysiological characteristics of the said varieties, including at the early phases of ontogenesis, starting from forming and development of cotyledon leaves.

When grown in water culture (control), root length of seedlings of both varieties was almost the same and increased throughout the period of study (Table 1). By phase 3, it increased by 57-60% vs. phase 1. It indicates the considerable similarity in the initial stages of growth of underground organs of the seedling of both buckwheat varieties [1].

Forming and development of aboveground organs is supported by both endogenous stockpile of metabolites and forming of new metabolites through photosynthesis [2, 27]. In our experiments, the height of hypocotyls in the Devyatka seedlings throughout all ontogenesis phases significantly exceeded the same in Dasha variety (by 16% on the average, $p \leq 0.05$). In both varieties, their increase was observed on transition to the 2nd phase (by 40% vs. 1st phase), whereafter the hypocotyl height did not change.

The data obtained correlates with hypocotyl weight which in Devyatka variety seedlings was significantly ($p \leq 0.05$) higher than in Dasha variety. At the same time, in Devyatka variety it remained unchanged during the first two phases of ontogenesis, and by the 3rd phase it increased by 19% ($p \leq 0.05$). In Dasha seedlings, hypocotyl weight did not change throughout the growth period. These results are the evidence of the faster growth and gaining of biomass in overground organs of traditional variety (Devyatka) seedlings as compared to the next-generation variety (Dasha).

Forming and development of leaves enables the plants to switch to autotrophic nutrition type [1, 28]. At all ontogenesis stages, the weight of cotyledon leaves of seedlings of both varieties was small and virtually the same. The only exceptions were the Devyatka seedlings in which at the 3d phase the cotyledon leaf weight was 33% higher ($p \leq 0.05$) (see Table 1).

1. Age-dependent morphophysiological characterization of seedlings of two buckwheat (*Fagopyrum esculentum* Moench) varieties under different growth conditions (M±SEM, lab experiment)

Ontogenesis phase	Root length, cm	Hypocotyl		Cotyledon leaf weight, g	Cotyledon leaf water content, %
		height, cm	weight, g		
Control (water)					
Devyatka variety					
1st	7.08±0.30^c	9.85±0.41^d	0.13±0.02^d	0.04±0.004^d	89.53±1.62^a
2nd	11.67±0.44^b	14.07±0.31^b	0.14±0.02^d	0.04±0.009^d	91.07±0.37^a
3d	12.04±0.80^a	14.32±1.12^b	0.16±0.02^c	0.06±0.006^c	91.05±0.16^a
Dasha variety					
1st	7.42±0.32^c	8.27±0.46^e	0.11±0.01^e	0.04±0.003^d	88.77±0.69^a
2nd	11.85±0.44^b	11.74±0.31^c	0.11±0.01^e	0.04±0.005^d	91.24±0.17^a
3d	13.00±1.00^a	11.97±1.07^c	0.12±0.01^e	0.04±0.004^d	91.43±1.45^a
Hoagland-Arnon nutrient medium (test)					
Devyatka variety					
1st	7.62±0.44^c	9.11±0.87^d	0.15±0.01^c	0.06±0.006^c	88.28±0.11^a
2nd	8.20±0.50^c	14.69±0.91^b	0.21±0.02^b	0.06±0.010^c	91.96±0.29^a
3d	11.02±0.26^b	17.97±0.21^a	0.27±0.02^a	0.08±0.013^a	93.50±0.60^a
Dasha variety					
1st	7.72±0.38^c	4.75±0.50^f	0.14±0.02^d	0.05±0.005^c	88.79±0.50^a
2nd	8.26±0.44^c	13.69±0.29^b	0.21±0.01^b	0.07±0.007^b	91.93±0.27^a
3d	11.24±0.32^b	17.21±0.59^a	0.27±0.02^a	0.07±0.013^b	95.48±1.58^a

Note. Statistically significant differences of mean values at $p \leq 0.05$ are marked with different Latin characters.

On Hoagland-Arnon nutrient medium (experiment), i.e. under the conditions of provision with macro- and microelements, development of seedlings speeded up against control group. In morphophysiological characteristics, 6-day old seedlings in test group corresponded to 11-day old seedlings in control group, 11-day olds corresponded to 14-day olds, 14-day olds corresponded to 18- day olds.

Root length of the seedlings of the two varieties did not differ at any stages of study. However, during the 2nd and 3rd phases, it was lesser than in control group (see Table 1). Root growth throughout the period made 30%, i.e. availability of macro- and microelements in the environment slowed down the development of underground organs.

Hypocotyl height in Devyatka variety seedlings during the 1st and 2nd phases of ontogenesis was the same in test and control variants, and during the 3rd phase it was larger in the experimental samples. In Dasha variety, the experimental variant always significantly ($p \leq 0.05$) differed from control group; during the 1st phase, the values were lower, during the 2nd and 3rd phases they were higher. It should be also noted that hypocotyl height in Devyatka variety seedlings was 50% smaller than in Dasha seedlings during the 1st phase, and later it became almost equal. Its total increase throughout the study period made 50% in Devyatka variety and 72% in Dasha variety. As for the hypocotyl weight, it was almost the same in both varieties, and over the seedling growth period increased by 44%. Its values throughout all phases in the experimental variant exceeded the control.

Measuring the weight of cotyledon leaves of the seedlings of the two buckwheat varieties showed no significant differences between them. In the process of ontogenesis, it increased by 25% in Devyatka variety and by 28% in Dasha variety. In general, almost all indicators of aboveground organs in the experimental variant seedlings, especially at the final stage of study (the 3d phase), were significantly ($p \leq 0.05$) higher than those of control group, which evidences the stimulating effect of nutrient solution.

Tissue water content is an important indicator for evaluation of physiological state of plants [1]. Water content in cotyledon leaves of seedlings of the two buckwheat varieties was the same and was increasing during ontogenetic development (see Table 1). The highest values were registered during the final growth phase. Provision of buckwheat seedlings with mineral nutrients did not affect this value.

2. Age-dependent pigment content in cotyledon leaves of the seedlings of two buckwheat (*Fagopyrum esculentum* Moench) varieties under different growth conditions (*M*±SEM, lab experiment)

Ontogenesis phase	Chlorophylls, mg/g dray weight			Chlorophylls, *a*/*b*	Carotenoids, mg/g dray weight
	a	*b*	*a* + *b*		
			Control (water)		
			Devyatka variety		
1st	5.29 ± 0.28^{d}	1.24 ± 0.12^{e}	6.53 ± 0.40^{d}	4.26	0.81 ± 0.04^{d}
2nd	6.19 ± 0.05^{c}	1.51 ± 0.04^{d}	7.70 ± 0.09^{c}	4.09	0.98 ± 0.03^{c}
3d	5.67 ± 0.87^{cd}	1.48 ± 0.26^{d}	7.15 ± 1.13^{c}	3.83	0.49 ± 0.05^{f}
			Dasha variety		
1st	5.14 ± 0.18^{d}	1.30 ± 0.11^{e}	6.44 ± 0.29^{d}	3.95	0.71 ± 0.10^{e}
2nd	5.25 ± 0.42^{d}	1.38 ± 0.12^{de}	6.63 ± 0.54^{d}	3.80	0.96 ± 0.09^{c}
3d	5.14 ± 0.68^{d}	1.39 ± 0.04^{de}	6.53 ± 0.72^{d}	3.70	0.52 ± 0.19^{f}
			Hoagland-Arnon nutrient medium (test))		
			Devyatka variety		
1st	2.28 ± 0.02^{f}	3.51 ± 0.05^{a}	5.79 ± 0.07^{e}	0.01	–
2nd	5.44 ± 0.18^{d}	1.49 ± 0.16^{d}	6.93 ± 0.34^{d}	3.65	0.50 ± 0.04^{f}
3d	7.25 ± 0.03^{b}	1.90 ± 0.15^{c}	9.15 ± 0.18^{B}	3.81	1.23 ± 0.01^{b}
			Dasha variety		
1st	3.19 ± 0.09^{e}	3.78 ± 0.26^{a}	6.97 ± 0.35^{d}	0.84	–
2nd	6.22 ± 0.53^{c}	1.65 ± 0.12^{d}	7.87 ± 0.65^{c}	3.76	0.65 ± 0.08^{e}
3d	9.09 ± 0.61^{a}	2.39 ± 0.17^{b}	11.48 ± 0.70^{a}	3.80	1.45 ± 0.04^{a}

Note. Statistically significant differences of mean values at $p \leq 0.05$ are marked with different Latin characters. Dashes mean that carotenoids were found starting from the 2nd phase of ontogenesis.

Plant photosynthesis is the main biological process supporting the life of all organisms on the planet [27]. Its effectiveness is evaluated by content of *a* and *b* chlorophylls and their ratio in leaves [28]. Under control conditions of our experiments, we have found differences in accumulation of *a* and *b* chlorophylls in cotyledon leaves as the seedlings of both buckwheat grew (Table 2). In Devyatka variety, *a* chlorophyll content during the 1st phase was the least, during the 2nd phase significantly increased by 17% ($p \leq 0.05$), and by the 3rd phase reduced by 10% but still exceeded that of the 1st phase. The similar, but less pronounce tendency was found in respect of *b* chlorophyll. In cotyledon leaves of Dasha variety seedlings, content of *a* and *b* chlorophylls throughout the study period did not change and was almost the equal to the same in Devyatka variety seedling in the 1st phase.

In assessment of photosynthetic productivity of plant tissues, it is important to take into account the *a* to *b* chlorophyll ratio which, in optimal growing conditions, approaches 3 [27, 28]. For cotyledon leaves of seedlings of both buckwheat varieties in the control group, higher *a* to *b* chlorophyll ratios were registered, and to the larger extent it was typical for 1st and 2nd phases (see Table 2).

Plant pigment system, in addition to chlorophylls, contains carotenoids which participate in functioning of reaction centers and light-harvesting complexes of chloroplast photosystems, absorb light in blue spectrum, protect photosynthetic apparatus from photodestruction and perform other protective functions [29, 30]. In both buckwheat varieties, similar tendencies in accumulation of carotenoids were observed, i.e. high content during the 1st phase, further increase during the 2nd phase (approximately by 20%) and considerable decrease during the 3rd phase (almost double). Hence the initial stages of forming and development of cotyledon leaves in buckwheat seedlings at low level of mineral nutrition are characterized by considerable accumulation of carotenoids, which may be the evidence of their important role during this period of ontogenetic development [31].

Study of pigment accumulation in cotyledon leaves of buckwheat seedlings grown on nutrient medium has revealed somewhat different tendencies. The content of *a* and *b* chlorophylls in them significantly ($p < 0.05$) grew throughout the observation period, which was not typical for control group (see Table 2). During the 1st phase, the amount of *a* chlorophyll in cotyledon leaves of Devyatka and Dasha seedlings was minimal (2.3 and 1.6 times, respectively, lower than in control group, $p \leq 0.05$). *b* chlorophyll content during this phase was the largest, and was almost 3 times the control value ($p \leq 0.05$). Further development of cotyledon leaves (2nd and 3rd phases) were accompanied by significant ($p \leq 0.05$) increase of *a* and *b* chlorophylls in them, which was to the larger extent manifested in Dasha variety. In its breeding, the selection for photosynthetic productivity of plants was conducted [27], and this feature has manifested even at the earliest stages of their development. It should also be noted that the total content of *a* and *b* chlorophylls in cotyledon leaves in experimental variants throughout the study period increased significantly ($p \leq 0.05$), i.e. by 58% in Devyatka variety and by 64% in Dasha variety.

As for *a* to *b* chlorophyll ratio in cotyledon leaves, during the 1st phase it was low (0.06 and 0.80 in Devyatka and Dasha varieties, respectively), and during the 2nd and 3rd phases increased considerably and became almost equal for both varieties (3.76 on the average). These values corresponded to control group, i.e. there was a considerable similarity in forming photosynthetic apparatus in buckwheat cotyledon leaves during the later ontogenesis phases which did not depend on mineral nutrition of seedlings (see Table 2).

In the experimental variants, accumulation of carotenoids in cotyledon leaves was almost the same in the seedlings of the both buckwheat varieties (see Table 2). It was registered starting from phase 2, but it was lower than in control group, and by phase 3 it increased almost 2.5 times ($p \leq 0.05$) and considerably exceeded the control.

In general, availability of nutrients promoted the effective formation of chlorophylls and carotenoids, which was the result of rapid growth of plants, development of cotyledon leaves and forming of chloroplasts, the important sources of energy and metabolites [27, 28].

It is well-known that during the initial ontogenesis phases, the plants need considerable energy and metabolites for growing and building up biomass [1, 2]. Soluble sugars become the main transport form for assimilates and may serve as initial substrates for many metabolic processes and formation of structural elements of cells and tissues, which is necessary for seedling development [32].

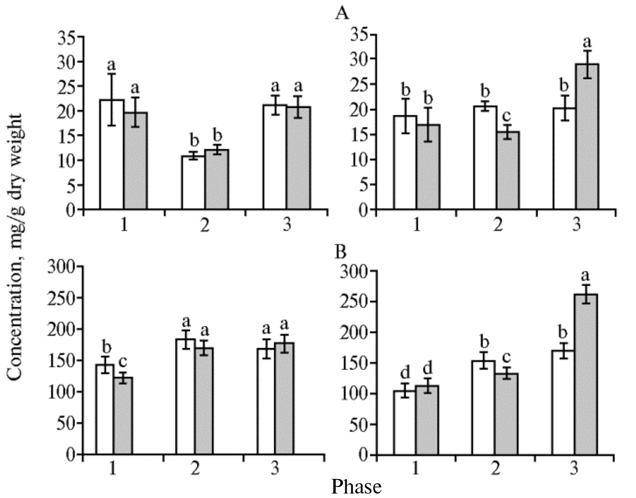

Fig. 1. Content of sugars (A) **and phenolic compounds** (B) **in cotyledon leaves of buckwheat (*Fagopyrum esculentum* Moench) Devyatka** (white bars) **and Dasha** (gray bars) **variety seedlings grown on water** (left) **and Hoagland-Arnon nutrient medium** (right) **during ontogenesis**. Significant differences of mean values at $p \leq 0.05$ are denoted by different Latin characters above the bars.

Accumulation of soluble sugars in cotyledon leaves in control groups was almost the same in the seedlings of the two buckwheat varieties over three phases of ontogenesis (Fig. 1, A). For the 1st phase, the amount of sugars was high, for the 2nd phase it significantly decreased (by 51% at $p \leq 0.05$), during the 3rd phase it increased ($p \leq 0.05$) and reached the initial values. The differences were caused by provision with initial substrates for formation of soluble sugars, namely spare substances in buckwheat seeds (1st phase), their subsequent depletion and active growth of seedlings (2nd phase) and finally by photosynthesis (3rd phase) resulting in increase in metabolite concentration and accumulation of soluble sugars [23, 32].

For accumulation of soluble sugars in cotyledon leaves of seedlings grown on nutrient medium, another tendency was observed (see Fig. 1, A). In Devyatka variety, in all stages of the study the content of soluble sugars was the same and sufficiently close to the control group during 1st and 3rd phases. In Dasha variety, it was equal to that in Devyatka variety during the 1st phase, decreased by 25% ($p \leq 0.05$) during the 2nd phase, and increased by 47% ($p \leq 0.05$) during the 3rd phase. Such pattern is only typical for Dasha variety which had been created by breeders through selection for photosynthetic activity [20, 26].

Phenolic compounds are ones of the most important plant metabolites, whose roles, just as chemical structure, are extremely diverse [6, 7]. When growing under control conditions, during the 1st phase the amount of phenolic compounds in Devyatka variety cotyledon leaves exceeded that of Dasha variety by 13% (see Fig. 1, B). During the 2nd and 3rd phases, the accumulation of phenolic compounds significantly ($p \leq 0.05$) increased in Devyatka and Dasha varieties by 22% and 28%, respectively, and became the same. To a certain extent it could be the cause of equal photosynthetic activity of cotyledon leaves during the said ontogenesis period, which is evidenced by content of photosynthetic pigments in them (see Table 2). It is known that chloroplasts are one of the main spots of biosynthesis of phenolic compounds in green plant cells [33].

When grown on nutrient medium, total content of phenolic compounds in cotyledon leaves of the seedlings of both buckwheat varieties in the majority of cases was significantly ($p \leq 0.05$) lower than in control group (see Fig. 1, B). In Devyatka variety, the least value was registered during the 1st phase, by the 2nd phase it increased by 32% ($p \leq 0.05$) and remained at that level until the 3rd phase, just as in control group. In Dasha variety, the amount of phenolic compounds during the 1st phase also was the least and did not differ from that of Devyatka variety. By the 2nd phase it significantly increased by 15% ($p \leq 0.05$), and by the 3rd phase it doubled and reached its maximum value.

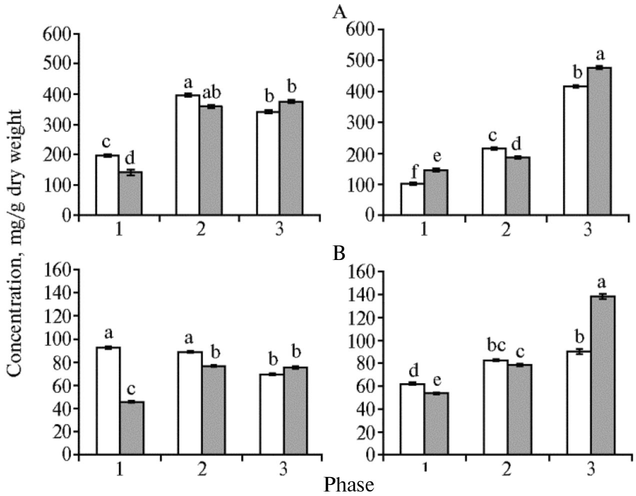

Fig. 2. Phenylpropanoid (A) **and flavonoid** (B) **content in cotyledon leaves of buckwheat (*Fagopyrum esculentum* Moench) Devyatka** (white bars) **and Dasha** (gray bars) **variety seedlings grown on water** (left) **and Hoagland-Arnon nutrient medium** (right) **during ontogenesis**. Statistically significant differences of mean values at $p \leq 0.05$ are denoted by different Latin characters above the bars.

As it was mentioned before, phenolic compounds in the plants are extremely diverse in their structure and are represented by different classes [7]. The simplest of them are phenylpropanoids [6]. In cotyledon leaves of buckwheat seedlings grown under control conditions, the content of phenylpropanoids during the 1st phase was the least, which to the large extent was manifested in Dasha variety (Fig. 2, A). By the 2nd phase, it became significantly ($p \leq 0.05$) larger (in Devyatka and Dasha varieties by 50% and 60%, respectively), and in future (3rd phase) decreased in Devyatka variety by 14% ($p \leq 0.05$) and remained unchanged in Dasha variety. The result was the same content of phenylpropanoids in cotyledon leaves of the seedlings of both buckwheat varieties by the end of study period.

With sufficient mineral nutrition, accumulation of phenylpropanoids in cotyledon leaves during the 1st phase and especially during the 2nd phase was lower as compared to control, and during the 3rd phase exceeded it (see Fig. 2, A). During the 1st phase, this value was the least, which to the larger extent manifested in Devyatka variety. By the 2nd phase, it significantly ($p \leq 0.05$) increased (in Devyatka and Dasha varieties by 53% and 22%, respectively). The largest changes in phenylpropanoid content were registered during the 3rd phase when their values increased sharp (by 50% on the average, $p \leq 0.05$) and became sufficiently close to those of the control group.

It is known that it is typical for the buckwheat to form flavonoids, the most widespread phenolic compounds in overground organs of the plants [6, 16]. When grown on water, the flavonoid content in cotyledon leaves of Devyatka variety was almost twice that value in Dasha variety during the 1st phase of ontogenesis. During the 2nd phase it did not change, and during the 3rd phase it decreased by 22% ($p \leq 0.05$). Another trend was observed in Dasha variety: flavonoid content increased by the 2nd phase by 41% ($p \leq 0.05$) and did not change further.

When grown on nutrient medium, flavonoid content in buckwheat seedling cotyledon leaves differed from control values in both varieties (see Fig. 2, B). In Devyatka variety it increased by 25% ($p \leq 0.05$) during the 1st and 2nd phases whereafter it remained the same, while in Dasha variety the increase was observed throughout the study period (by 32% for the 1st and the 2nd phase, and by 43% for the 3rd phase; $p \leq 0.05$).

All of the above demonstrates that the mineral nutrition conditions considerably affect the initial ontogenesis phases of buckwheat plant. When grown on Hoagland-Arnon nutrient medium, the rate of growth of overground organs and accumulation of pigments (*a* and *b* chlorophylls and carotenoids) in cotyledon leaves was higher as compared to control group, being to the larger extent manifested by the end of the study period (3rd ontogenesis phase). There are reports on the positive effects of mineral nutrition on building up of biomass of plants and content of different forms of chlorophyll in plant leaves [22, 34, 35]. As for *a* to *b* chlorophylls ratio, this indicator did not depend on the mineral nutrition conditions.

Sugars are the intermediate products of photosynthesis [28]. Positive correlation was fairly often registered between their accumulation and chlorophyll content [28, 32]. However in case of seedlings of two buckwheat varieties, no clear tendency was observed. Total content of phenolic compounds in cotyledon leaves of control group was higher than in the test group. Thus, under the conditions of better provision of buckwheat seedlings with mineral elements, the amount of secondary phenolic metabolites in the aboveground organs decrease, which may the result of their intensive growth. There were reports of decrease in accumulation of polyphenols as the plant growth activated [6, 9]. The content of their certain classes (phenylpropanoids and flavonoids) in cotyledon leaves of Dasha seedlings grown on nutrient medium normally increased, while in Devyatka seedlings it decreased, except for the 3rd growth phase, when an opposite tendency occurred. Changes in biosynthesis of certain classes of phenolic compounds in buckwheat seedlings under different mineral nutrition conditions require further research.

Thus, the initial stages of ontogenetic development of the two buckwheat varieties seedlings are defined by the stockpile of nutrients in the seeds and availability of micro- and macroelements. Introduction of the latter speeds up the growth of overground organs (by 20-30% on the average), development of cotyledon leaves (by 25% for Devyatka variety and by 42% for Dasha variety) and enhances photosynthetic activity as compared to the similar indicators of seedlings grown on water instead of nutrient medium. This affects the pigment content, accumulation of primary (sugars) and secondary (various classes of phenolic compounds) metabolites. Therefore, changes in provision with mineral nutrition elements during plant cultivation enable the regulation of their growth and development and metabolite accumulation.

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