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FEATURES OF THE PHENOLICS' FORMATION IN SEEDLINGS OF DIFFERENT VARIETIES OF BUCKWHEAT (*Fagopyrum esculentum* Moench)

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Abstract

A unique feature of higher plants is the capacity to form phenolic compounds, the substances with high antioxidant activity. These secondary metabolites play an important functional role, including cell and tissue protection against stress factors. It is especially important at the initial stages of plant ontogenesis. Buckwheat (*Fagopyrum esculentum* Moench) is the major cereal crop for which the formation of various phenolics, particularly rutin, a biologically active compound of plant origin successfully used in pharmacology, is characteristic. In the young seedlings (at the age of 14 days) of 10 buckwheat varieties mostly bred in Russian research centers during recent decades we studied the morphophysiological parameters and the accumulation of phenolic compounds, including phenylpropanoids and flavonoids, in the hypocotyls and cotyledons. Particularly, the highest level of phenylpropanoids was found in Bol'shevik 4 and Bashkirskaya krasnostebel'naya varieties. Note, it was high and almost equal in the hypocotyls and cotyledons. In the rest varieties the amount of phenylpropanoids in seedlings was 20 to 50 % lower, and in the cotyledons it was 1.5-2.0 times higher compared with the hypocotyls. Accumulation of flavonoids was higher in the Dialog variety, somewhat lower in Bol'shevik 4 and Bashkirskaya krasnostebel'naya varieties (by 10 and 17 %, respectively), and 35 to 40 % lower in the other studied forms. The highest content of anthocyanins was shown in the Bashkirskaya krasnostebel'naya plants, while in other studied buckwheat varieties it was lower. Particularly, in Devyatka, Bol'shevik 4 and Temp plants a decrease was about 50 %, and in Batyr, Dialog, Chatyr tau, Ilishevskaya, Dizain, and Dikul' varieties 70 to 80 % decrease was found. It was shown that recently bred buckwheat varieties have a very rapid growth and development of seedlings, which is important for their better adaptation in the early ontogeny. The most promising varieties, along with Bashkir krasnostebelnaya, are Bolshevik 4, Nine, Dialogue and Tempo. They are characterized by a high capacity for accumulation the phenolic compounds, the important components of the antioxidant defense system in plants. This feature of their metabolism may be a potential criterion for plant resistance to stress factors

Keywords: buckwheat, *Fagopyrum esculentum* Moench, varieties, phenolic compounds, phenylpropanoids, flavonoids, anthocyanins.

Buckwheat is the major cereal food crop in the world [1]. Russia, China and some other countries are the world leaders in buckwheat production [2, 3] with the crop area about 2.5 million hectares. In Russia buckwheat is mainly cultivated in Volga region, Central Chernozem region, Bashkortostan, Tatarstan, West and East Siberia. *Fagopyrum esculentum* Moench, a widely grown buckwheat species, is peculiar in high accumulation of fenolics [4, 5]. Buckwheat leaves are raw material for commercial production of rutin which possesses antioxidative, angioprotective, antibacterial and hepatoprotective activity being an ingredient of a number of medicinal preparations [1].

At that, the buckwheat plants are less competitive compared to other groat crops due to specific biological features such as long flowering, unsynchronized maturation in the crops, sensitivity to low temperatures during early ontogenesis, etc. [6]. So breeding new varieties with tolerance to abiotic and biotic stresses have been widely carried out, when selected plants were mostly estimated morphophysiologically, with regard to resistance to lodging and pathogens, high yield and grain quality [7, 8] while production of phenolic compounds in buckwheat plants is poorly studied [4, 9].

Phenolics are secondary metabolites produced mainly in higher plants [10, 11]. Phenolic compounds are numerous and extremely different in structure and chemical properties. There are phenylpropanoids, in particular hydroxycinnamic and hydroxybenzoic acids, and flavonoids (flavones, flavonols, anthocyanins, etc.). Phenolic compounds are formed in all plant tissues and their functional role is extremely varied and associated with the processes of photosynthesis, respiration, allelopathy, anti-stress effects, regulation of plant growth and development [10, 12]. The accumulation of these secondary metabolites depends on the plant species, stage of development and growth conditions [11, 13]. The study of the formation of polyphenols is of great practical interest, since they play an important role in the regulation of plant life. Furthermore, they have a high biological and antioxidant activity that makes their practical use successful. Currently, these secondary metabolites of higher plants, including the so-called bioflavonoids, are of great interest throughout the world with regard to their biosynthesis and its regulation, on one side, and the role in plant adaptation to stresses, on the other side [11, 14]. Much attention is also paid to the antioxidant activity of various compounds of phenolic nature and their use in medicine and pharmacology [15-17].

We have studied the morphophysiological characteristics of seedlings in 10 buckwheat varieties bred in the latter years mainly as related to the accumulation of different classes of phenolics, including flavonoids. This approach allows us to determine the peculiarities of their synthesis at early ontogenesis. This aspect is very important because during this period the buckwheat seedlings are exposed to different stresses such as low temperature, low humidity, etc., which leads to their death and, as a consequence, to a decrease in the crop yield. Due to estimation of endogenous levels of polyphenols in this period it is possible to presume a potential stability of plants, which is of practical importance.

Technique. We studied 10 buckwheat (*Fagopyrum esculentum* Moench) middle-ripening varieties bred mostly not long ago, with different heat and drought resistance. The varieties have been included in the State Register of breeding achievements of the Russian Federation. Seed samples were obtained from the collection of All-Russian Research Institute of Legumes and Groat Crops, except Bol'shevik 4 variety from the collection of Kropotovskaya biostation of N.K. Kol'tsov Institute of Developmental Biology RAS. Seeds were kept in water for 24 hours without lightening, then placed into filter paper rolls and grown in phytotron chamber (K.A. Timiryazev Institute of Plant Physiology RAS) for 14 days at 24 °C with 16 hour photoperiod. In seedlings the stem height, root length, and the weight of cotyledons were evaluated. Water content in tissues was estimated after drying at 70 °C to constant weight.

Phenolic compounds were extracted from cotyledons and hypocotyls with 96 % ethanol. The presence of different classes of phenolic compounds in supernatant was analyzed spectophotometrically. Total soluble phenolic compounds were determined at λ = 725 nm with the Folin and Denis reagent, and flavonoids were assayed at λ = 430 nm using 1 % $AlCl_3$ water solution [18, 19]. Calibration curves for rutin was used in both cases (Chemapol, Czech Republic) with the tested levels expressed as rutin milligram equivalents (ME) per 1 g dry weight. The level of pheniylpropanoids was measured directly in ethanol extracts at λ = 330 nm [20] using calibration curve for caffeic acid (Serva, Germany) with the tested compounds expressed as coffeic acid (ME) per 1 g dry weight. Anthocyanins were extracted from lower parts of hypocotyls using 3 % HCl in ethanol with direct spectophotommetry of the supernatant at λ = 525 nm [21]. Based on the calibration curves for cyaniding (Sigma, USA) the anthocyanin concentration was considered as equal amount of cyaniding (ME) per 1 g dry weight. A spectrophotometer SF 46 (Russia) was used.

Experiments were carried out in 3-fold biological and 3-fold analytical replicates. For obtained data processing the Statistica software were used. The figures show the average values and their standard deviations.

Results. The varieties involved in this investigation (Table 1) are due to attention paid by Russian researchers to theoretical background and practical use of buckwheat genetic systems in breeding programs.

1. Buckwheat (*Fagopyrum esculentum* Moench) Russian bred varieties involved in the study

Originator	Variety	Included in the State Register of the RF
Institute of Developmental Biology RAS, Moscow	Bol'shevik 4	1963
All-Russian Research Institute of Legumes and Groat Crops, Orel	Dikul'	1999
	Dialog	2008
	Dizain	2010
	Temp	2010
Tatar Research Institute of Agriculture, Kazan'	Devyatka	2004
	Chatyr Tau	2005
Bashkir Research Institute of Agriculture, Ufa	Ikishevskaya	2008
	Bashkirskaya krasnostebe'naya	2009

The highest levels of flavonoids is peculiar to Bashkirskaya krasnoste-bel'naya variety selected from a hybrid population produced by crossing red-flowered Rubra variety mutant and Chernoplodnaya, Ufimskaya and Chishmin-skaya varieties [8, 22]. As to another varieties, data are rare [4, 9] with estima-tion conducted mostly at late ontogenesis. The initial stages of buckwheat plant development are extremally poor studied with regard to growth and accumula-tion of polyphenols.

Data shown in the Table 2 indicates insignificant morphophysiological differences in juvenile plants between the varieties.

2. Morphometric parameters ($X \pm x$) of seedlings in different buckwheat (*Fagopy-rum esculentum* Moench) varieties (laboratory tests)

Variety	Height, sm	Root length, sm	Weight of cotyledons, g
Bashkirskaya krasnostebe'naya	10.01±0.55	13.90±0.70	0.067±0.008
Dikul'	11.30±0.41	12.70±0.58	0.062±0.009
Bol'shevik 4	10.70±0.31	13.70±0.67	0.056±0.007
Batyr	11.43±0.25	11.04±0.87	0.062±0.008
Devyatka	13.10±0.38	11.20±0.59	0.063±0.005
Dialog	10.56±0.40	10.01±0.50	0.048±0.009
Dizain	12.30±0.30	11.00±0.83	0.073±0.008
Ilishevskaya	12.70±0.47	9.41±0.76	0.090±0.006
Tenp	12.80±0.33	10.30±0.96	0.054±0.009
Chatyr Tau	11.36±0.36	9.50±0.88	0.049±0.008

N o t e. For detail (origin of the varieties and time when they have been included into State Register of the RF) see Table 1 and text.

The seedlings of Devyatka, Ilishevskaya and Temp varieties were the highest, and the seedlings of Bashkirskaya krasnostebel'naya and Dialog varieties were shortest. Minimal root length was observed in Ilishevskaya and Chatyr Tau varieties while in Bol'shevik 4 and Bashkirskaya krasnostebel'naya seedlings the root length was maximal. In all variants differences reached 30 %.

Note, as plant height decreased, the tendency to root length increase was traced, being more pronounced in Bashkirskaya krasnostebel'naya, Dikul' and Bol'shevik 4 plants, and, otherwise, the higher plants, the shorter roots were, particularly in Devyatka, Dizain, Ilishevskaya, Temp and Chatyr Tau plants. There were only Batyr and Dialog plants in which these parameters were in fact identical. Besides, in the buckwheat varieties originated in 2008-2010 more intensive growth was characteristic if compared to other varieties. This is important because the buckwheat plants in the early ontogeny are very sensitive to stress factors [3]. Accelerate growth and hence more rapid formation and development of cotyledon leaves allows seedlings to change rapidly the type of supply to autotrophic that provides them better survival and adaptation.

Cotyledon weight was the greatest in the Ilishevskaya seedlings (see Table 2) being lower in other varieties, e.g. by 20-23 % in Bashkirskaya krasnostebel'naya plants, by 28-30 % in Devyatka, Dikul'and Batyr plants, and by 40-53 % in temp, Catyr Tau and dialog varieties. These results are indicative of the differences in the formation and development of the cotyledons in the early ontogenesis of buckwheat seedlings.

An important indicator in evaluating the physiological state of the plant tissues is their water content, which depends on the structure, the age and conditions of cultivation of plants [23]. It is known that the water content of the leaves reaches a maximum at the beginning of the growing season, gradually decreasing toward its end [24]. The water content in cotyledon leaves of most varieties was 90 %, and only in the variety Dikul' it was higher reaching 93 %. For hypocotyls a little larger value (up to 95 %) occurred. This is probably due to the water-retaining capacity of the cells, while on the leaf surface the evaporation occurs due to stomata functions [25]. It should be emphasized that water content in hypocotyls and cotyledons in seedling did not depend on varietal specificity in buckwheat plants.

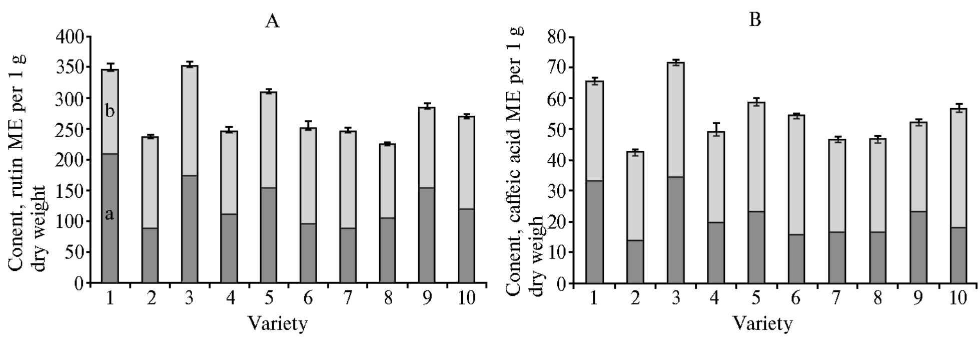

Fig. 1. Total phenolic compounds (A) **and phenylpropanoids** (B) **in hypocotyls** (a) **and cotyledons** (b) **in seedlings of different buckwheat (*Fagopyrum esculentum* Moench) varieties:** 1 — Bashkirskaya krasnostebe'naya; 2 — Dikul'; 3 — Bol'shevik 4; 4 — Batyr; 5 — Devyatka; 6 — Dialog; 7 — Dizain; 8 — Ilishevskaya; 9 — Temp; 10 — Chatyr Tau (laboratory experiments).

As already mentioned, the formation of different classes of phenolic compounds is characteristic for buckwheat [1, 4, 9]. Their total content, which reflects the biosynthetic capacity of plant tissues, showed the greatest indexes in young seedlings of the varieties Bashkirskaya krasnostebel'naya and Bol'sheviks 4 (Fig. 1, A). In the first case, it was due to higher level in hypocotyls compared with leaves, and in this almost 50 % difference occurs. Such a distribution of phenolic compounds in the tissues of higher plants is rare. Probably, it was formed as a result of breeding varieties Bashkirskaya krasnostebel'naya aimed at making buckwheat with a high capacity for the biosynthesis of secondary metabolites [8]. A similar pattern of phenolic compounds' distribution, but expressed to a much lesser extent, can be traced in seedlings of Temp varieties obtained after repeated and negative mass selection of a combination of Donor KKG × line BO 3-5. In addition, in the State Register of selection achievements in the Russian Federation, the accumulation of phenolic compounds in this variety is not reported, and only its resistance to lodging and drought are mentioned. Thus, the Temp variety deserves attention, and its study should be continued.

In the variety Bilshovyk 4 the level of phenolic compounds was high and almost equal in the hypocotyls and the cotyledons. A similar trend was observed in seedling varieties Devyatka, although the total content of phenolic compounds was lower (by 13 % compared with the variety Bolshevik 4). In all other cases, a higher accumulation of these substances in the cotyledon leaves compared with hypocotyls were observed. The significant accumulation of phenolic compounds in the leaves, especially during the early stages of plant development, was reported [13, 26], that is to be expected, since the biosynthesis of phenolic compounds in the cells of higher plants depends on the chloroplast functions [10, 27, 28].

The major phenolic compounds in higher plants are phenylpropanoids and flavonoids [10, 11]. Phenylpropanoids are biogenetically earlier phenolic substances of phenol metabolism. These are C_6-C_3-compounds which can be accumulated in plant tissues and(or) used in the biosynthesis of flavonoids (C_6-C_3-C_6-compounds).

Phenylpropanoids are widely distributed in higher plants and included in the complex of buckwheat polyphenols [1]. The highest level was recorded in the buckwheat seedling of Bol'shevik 4 and Bashkirskaya krasnostebel'naya varieties (see Fig. 1, B). Reported results were high in hypocotyls and cotyledons being almost the same in value. In other cases, the level of phenylpropanoids in seedlings was 20-50 % lower and in the cotyledons it was 1.5-2.5 times higher than that in hypocotyls.

The main phenolic compounds of above-ground organs of the plants are flavonoids, comprising flavons, flavonols, flavonones, anthocyanidins, etc. [10]. In buckwheat plants the flavonols (rutin, quercetin, kaempferol and morin) and anthocyanins were found [1, 4, 9].

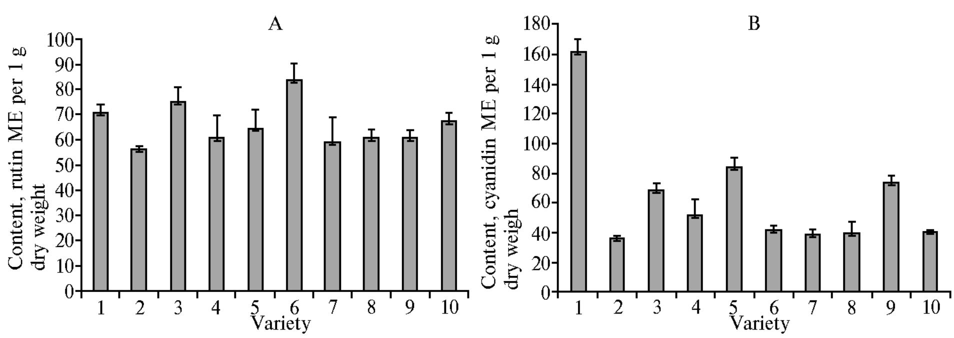

Fig. 2. Flavonoids in cotyledons (A) and hypocotyls (B) in seedlings of different buckwheat (*Fagopyrum esculentum* Moench) varieties: 1 — Bashkirskaya krasnostebe'naya; 2 — Dikul'; 3 — Bol'shevik 4; 4 — Batyr; 5 — Devyatka; 6 — Dialog; 7 — Dizain; 8 — Ilishevskaya; 9 — Temp; 10 — Chatyr Tau (laboratory experiments).

Accumulation of flavonoids in the cotyledon leaves were higher in buckwheat Dialog variety (Fig. 2, A), being slightly lower in Bol'shevik 4 and

Bashkirskaya krasnostebel'naya varieties (by 10 and 17 %, respectively) and much lower (by 35-40 %) in others.

Anthocyanins (pigments of higher plants) are phenolic substances [1, 10], which not only color organs of plants, but also participate in the protection of their tissues from stress factors (low temperature, heavy metal pollution, drought, etc.) [11]. Synthesis of anthocyanins is characteristic of the initial stages of the seedling development in buckwheat, and mostly for hypocotyl tissue (see Fig. 2, B). The greatest accumulation of anthocyanins was observed in Bashkirskaya krasnostebel'naya plants. It was lower by almost 50 % in Devyatka, Bol'shevik 4 and Temp plants, and by 70-80 % in Batyr, Dialog, Chater Tau, Ilishevskaya, Dizain and Dikul' plants

So, in the early ontogenesis the buckwheat varieties differ in morpho-physiology, distribution of phenolic compounds in the above-ground organs, and in the biosynthetic activity. Along with Bashkirskaya krasnostebel'naya variety, the Bol'shevik 4, Devayka, Dialog and Temp plants also may be deemed accumulating phenolic compounds. The high capacity of phenolic compounds accumulation as the major components of the plant antioxidant protective system can serve as a criterion of high plant resistance to stress factors.

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