ISSN 1022-7954, Russian Journal of Genetics, 2024, Vol. 60, No. 2, pp. 137–151. © Pleiades Publishing, Inc., 2024.
Russian Text © The Author(s), 2024, published in Genetika, 2024, Vol. 60, No. 2, pp. 00000–00000.

REVIEW
AND THEORETICAL ARTICLES

Adaptive Significance and Origin of Flavonoid Biosynthesis Genes in the Grain of Cultivated Cereals

A. N. Bulanov[a], * and A. V. Voylokov[b], **

[a] *Saint Petersburg State University, St. Petersburg, 199034 Russia*
[b] *Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, 119991 Russia*
**e-mail: an.bulanov20002014@gmail.com*
***e-mail: av_voylokov@mail.ru*

Received July 6, 2023; revised August 11, 2023; accepted August 23, 2023

Abstract—The majority of cultivated cereals including maize, rice, wheat, barley, oat and rye consist of numerous varieties lacking anthocyanin pigmentation or having weak coloration of vegetative organs and/or caryopses. Only rare local races and wild related species have intense coloration of plants and/or grains. The coloration of caryopses is associated with the biosynthesis of colored flavonoids in maternal (pericarp and testa) and hybrid (aleurone) caryopsis tissues. The trait is controlled by dominant alleles of regulatory genes encoding conserved transcription factors of the MYB, bHLH-MYC, and WD40 families forming the MBW protein complex. Recent studies have proven the participation of uncolored and colored flavonoids in the response of plants to biotic and abiotic stresses, and the significance of their presence in the whole grain foods has been determined. However, many questions about the adaptive effects and health benefits of anthocyanins remain unanswered. In particular, the reasons why the dominant alleles of regulatory genes controlling pericarp coloration did not become widespread in the course of domestication and breeding of cereals are not clear, although these genes receive special attention in association with health-improving effects of grain nutrition. This article discusses the similarity and specificity of the genetic control of the biosynthesis of flavonoids in the caryopsis in three related cultivated cereals, wheat, barley, and rye, and their biological role in the development of the caryopsis and seed germination.

Keywords: cereals, caryopsis, flavonoids, anthocyanins, genetic control
DOI: 10.1134/S1022795424020029

Normally, in cereals, the anthocyanin color of the plant, varying in intensity, appears on leaf blades and sheaths, ears, nodes and internodes, and awns and veins of glumes. Wheat and rye, unlike barley, are characterized by coleoptile coloration. The biosynthesis of anthocyanins in the vegetative organs of plants is significantly activated under the influence of biotic and abiotic stresses. Anthocyanins act as antioxidants that neutralize excess reactive oxygen species (ROS) formed during normal cell functioning and during the development of stress reactions [1, 2]. It is believed that anthocyanins are synthesized constitutively in generative organs—flowers, fruits, and seeds, attracting animal pollinators and seed distributors. However, this function of colored flavonoids is far from obvious in the case of cereals. Research conducted on corn [3] allowed us to conclude that almost any plant tissue with a normal genotype is capable of producing anthocyanins. The basic antioxidant potential of plant cells is provided by phenolic compounds present in colored and uncolored tissues, nonphenolic antioxidants (ascorbic acid and glutathione), and antioxidant enzymes that neutralize free radicals (superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase) [4].

The adaptive response is recorded on the basis of an increase in anthocyanin content or activation of transcription of anthocyanin biosynthesis genes in the vegetative organs of plants [5]. Various adaptive response mechanisms based on an increase in anthocyanin content are considered [5]. The first is related to the reaction to cold, salinity, and drought. In this case, the accumulation of anthocyanins restores the disturbed osmotic balance of cells. In the reaction of plants to heavy metal salts, the chelating properties of anthocyanins come to the fore. Cold, drought, and salinity, as well as heavy metal ions, nitrogen or phosphorus deficiency, high soil acidity, tissue damage, infection with many pathogens, intense lighting, and ultraviolet radiation, lead to excessive formation of reactive oxygen species that disrupt the structure of proteins, lipids, and nucleic acids. The universal mechanism of the adaptive response may be based on the high antioxidant capacity of anthocyanins, the synthesis of which is activated during the response to stress factors. The variety of options for the regulation

of anthocyanin biosynthesis, both at the level of one species and at the interspecific level, confirms their occurrence during adaptive evolution. Anthocyanins are some of the most active antioxidants among flavonoids and other compounds of primary and secondary metabolism [5]. Their role as antioxidants is also manifested in the outer tissues of the fruit in cereals with colored shells—the pericarp, testa, and aleurone layer. In each of these tissues, there is an evolutionarily developed mechanism for regulating the biosynthesis of colored flavonoids and mechanisms of their transport and accumulation in vacuoles. In wheat, barley, and rye with a purple (violet) color of the pericarp, the final products of biosynthesis are cyanidin derivatives, with a blue color of the aleurone—delphinidin derivatives. In the seed coat (testa) of wheat and rye, colored proanthocyanidins accumulate, and in barley, uncolored (unoxidized) proanthocyanidins accumulate. In wild related species, there is genetic variation in regulatory genes that control flavonoid biosynthesis in these tissues. Generally, ancestral wild species are characterized by colored grains; this also applies to the ancestors of corn, rice, and sorghum [6]. However, each of the cultivated cereals has its own history of loss and acquisition of grain color genes. Despite the functional conservatism of structural and regulatory genes of biosynthesis flavonoids, in different species of monocotyledonous and dicotyledonous plants, there is specificity in its organization associated with the species' ecological and physiological characteristics. Metabolic features are characteristic of such well-studied cereals as corn, rice, wheat, and barley. Wheat and barley are evolutionarily the closest; they belong to the Triticeae tribe. Rye also belongs to this tribe, research on which is in its infancy. Genome sequencing in rye opens up new prospects in the study of anthocyanin biosynthesis in this object in comparative terms and, first of all, in relation to the tissue-specific synthesis of flavonoids in grain [7, 8]. Grain shells play an important role during the development and germination of cereal seeds. The pericarp performs protective, photosynthetic, and transport functions, the aleurone ensures the accumulation and utilization of reserve substances, and the seed coat controls seed germination. Each of these shells has its own system for the biosynthesis of flavonoids, which have the function of protecting against environmental stress factors. Highly conserved elements of this system include structural biosynthesis genes encoding enzymes of the phenylpropanoid biosynthesis pathway [9]. The work of structural genes is controlled by regulatory genes encoding the transcription factors R2R3-MYB and bHLH-MYC, as well as the coregulator WD40. Together they form the so-called MBW complex, which recognizes regulatory elements in the promoters of structural genes and activates their expression [10].

TRANSPORT AND ACCUMULATION OF FLAVONOIDS IN VACUOLES

Enzymes involved in the biosynthesis of flavonoids can form a multienzyme complex on the surface of the endoplasmic reticulum, the composition and final products of which depend on the activity of transcription factors operating in a particular tissue [9, 10]. Glycosylation, acylation, and methylation at certain positions of the carbon skeleton atoms impart stability and specificity to the synthesized products. Glycosylated forms of anthocyanidins (anthocyanins), flavonols, flavones, and proanthocyanidin precursors are transported to the central vacuole, where modification is completed and, in the case of proanthocyanidin precursors, polymerization occurs. The central vacuole in a plant cell performs the functions of storing, detoxifying, and degrading chemical compounds under normal and stress conditions. It has been established that glycosyltransferases and acyltransferases belonging to different phylogenetic lines act in the vacuole and cytoplasm [11]. Uncolored flavonoids are also found in the nucleus, chloroplasts, and mitochondria; in these organelles, they can play a predominantly protective role, and in the nucleus, they can also perform a regulatory role [5]. Cytological studies revealed two types of distribution of anthocyanins in the cells of pigmented tissues [12]. Anthocyanins can be evenly distributed in the vacuole or occur in the form of compact, intensely colored formations, named according to the presence of pre-vacuole compartments (PVC) in the cytosol and anthocyanic vacuolar inclusions (AVI) in the vacuole. The morphology of AVI depends on the plant species and the type of cells in which anthocyanin accumulation (condensation) occurs. Most AVIs have the structure of vesicles with homogeneous contents; some have a round shape with granular contents. In the former, a membrane is present that forms the vesicle; in the latter, no membrane is found, which may serve as a characteristic feature of vesicles of this type. It has been established that the "condensation" and packaging of anthocyanins in the AVI structure is facilitated by their specific structure. In this case, the key factors are hydroxylation of the B-ring, glycosylation in 3-*O* position, and aromatic acylation [12]. The distribution of anthocyanins in the cell may be associated with different methods of their delivery to the site of localization. Two main mechanisms of flavonoid transport are discussed [11]. According to the first, the transporter glutathione *S*-transferase (GST), localized in the cytoplasm and associated with the endoplasmic reticulum, binds flavonoids and delivers them to the tonoplast, where such complexes enter the vacuole using ATP-binding cassette transporters (ABC). GST performs transport and protective functions before entering the vacuole. Such indirect transport in vesicles can exist in parallel with direct transport, since loading of vesicles can be carried out by direct transport [11]. It is assumed that the loading of flavonoids into the lumen

of the endoplasmic reticulum during the formation of pre-vacuoles is carried out, as in the case of transport through the tonoplast, with the participation of membrane transporters MATE (multidrug and toxic compound extrusion transporter) and ligandin (GST). It is most likely that ABC transporters play a major role in anthocyanin transport, while MATE transporters are less common [11]. The presence of several MATE isoforms indicates their possible specificity toward certain flavonoids or their different intracellular localization. The specificity of interaction with transporter proteins is also determined by the position of side methyl or acyl groups in the flavonoid molecule. Targeted delivery of vesicles and their fusion with the membranes of other organelles is determined by membrane receptors [13].

Cytological data on the transport and accumulation of anthocyanins in the pericarp and other caryopsis membranes are extremely scarce. By analogy with model objects in dicotyledons, two models of anthocyanin transport into the vacuole in black rice are discussed [14]. The first is movement to the tonoplast in vesicles with subsequent entry of the vesicles into the vacuole through the mechanisms of autophagy. The second model assumes the participation of transporter proteins of both cytoplasmic localization and those embedded in the tonoplast. During the research, the gene *OsMATE34* (*Os08g0562800*) was identified, coding a protein of the family of MATE transporters, localized in the plasma membrane and tonoplast and similar to proteins involved in anthocyanin transport in other plants [14]. When localized in the plasmalemma, this protein ensures the release of compounds from the cell in exchange for the H+ ion. When localized in the tonoplast, on the contrary, it promotes the uptake of transported compounds owing to a pH gradient, the values of which are high (alkaline) in the cytoplasm and low (acidic) in the vacuole. The gene *OsMATE34* and the gene of glutathione-*S*-transferase *OsGSTU34* (*Os10g0395400*) are differentially expressed in black rice grains along with structural genes for anthocyanin biosynthesis *OsPAL*, *OsCHS*, *OsCHI*, *OsF3H*, *OsDFR*, *OsANS*, and *OsUFGT*/*Os3GT*, which indicates a unified regulation of these genes by the MYB-bHLH-WD40 (MBW) complex [14]. A similar conclusion was made by the authors of the publication regarding one of the genes that controls resistance to drought and oxidative stress. This gene belongs to a family of genes that control the transcription of glutathione peroxidase genes, which is of obvious interest in connection with the discussed antioxidant function of anthocyanins. It is most likely that anthocyanins in black rice grains enter the vacuole through the mechanism of autophagy of vesicles loaded with anthocyanins with the participation of transport protein MATE [14]. Anthocyanins in the pericarp of rice appear on the seventh day from fertilization, and somewhat later anthocyanins are found in the testa and aleurone. Anthocyanin biosynthesis genes and transporter protein genes *OsMATE34* and *OsGSTU34* in black rice grains have the same transcription profile. At the same time, in the colored leaves of black rice, along with the gene *OsGSTU34*, the gene *OsMRP15* belonging to the family of genes of ABC transporters is active. It is assumed that this gene is also involved in the transport of flavonols in the tissues of uncolored stigmas in the same forms of black rice [15]. In protoplasts isolated from pigmented stigmas of the same forms of black rice, anthocyanin fluorescence was detected when irradiated with light of 552 nm. This made it possible to identify anthocyanins diffusely dissolved in the vacuole and anthocyanins concentrated in the form of intensely luminescent bodies, located mainly in the cytoplasm adjacent to the tonoplast and partially localized in the vacuole itself. The size and shape of the bodies corresponding to the previously described [12] anthocyanin vacuolar inclusions (AVI) vary significantly from cell to cell and within cells. This fact may indicate dynamics in the organization and movement of AVI during the accumulation of anthocyanins in the vacuole. The authors conclude that the obtained data correspond to previously proposed models of the synthesis and transport of anthocyanins in dicotyledons and the possible transfer of this model to the biosynthesis of anthocyanins in rice tissues [14].

The mechanisms of anthocyanin accumulation in the tissues of grains and other organs of plants are characterized by both conservatism and species, tissue, and ontogenetic specificity. In photomicrographs of sections of immature grains of two lines of corn with anthocyanins only in the aleurone or only in the pericarp, living aleurone cells look uniformly filled with pigments with many intensely colored inclusions. Pericarp cells, which at the time of analysis have gone through the processes of cell death, are compressed into a narrow colored strip [16]. In cereals aleurone cells contain two types of vacuoles—lytic vacuoles and protein storage vacuoles [17]. Both types of vacuoles coexist with each other for a short time in the same cell and each dominates at different stages of development. The tonoplasts of these vacuoles are similar in the presence of transport proteins. In ripening seeds, lytic vacuoles transform into vacuoles that accumulate proteins; during germination, the reverse transformation occurs. The types of vacuoles are distinguished using marker proteins. Both types of vacuoles merge during seed germination to form a central vacuole. [17]. During the germination of rice grains, the aleurone secretes hydrolytic enzymes into the starchy endosperm, and vacuole fusion occurs to form a central lytic vacuole, followed by cell death. Rupture of the tonoplast and destruction of the central vacuole is a morphological manifestation of programmed death of aleurone cells [18]. It can be assumed that uncolored flavonoids and anthocyanins, released during the destruction of the tonoplast, perform protective func-

tions in relation to the sensitive compounds of cells used by the seedling for growth and development.

FUNCTIONS OF GRAIN SHELLS AND ADAPTIVE REACTIONS OF PLANTS

The three-layer pericarp (endo-, meso-, and epicarp) is formed from the maternal tissues of the ovary wall. During early caryopsis development, the mesocarp undergoes programmed cell death, and the endocarp transforms into photosynthetically active tissue consisting of layers of transverse and tubular cells [19]. The pericarp is a site of photosynthetic carbon fixation and, at the same time, a place of temporary accumulation of photosynthesis products. It is believed that, owing to the small number of stomata on the surface of the pericarp, the main source of carbon is not atmospheric carbon dioxide, but carbon dioxide formed during respiration [19, 20]. Photosynthetic activity of the caryopsis endocarp can account for up to 42% of the total activity of the ear [21]. It persists until the later stages of grain development, supplying tissues with oxygen and photosynthetic products, as well as facilitating the absorption of excess light energy during the grain filling period [22]. Programmed death of pericarp cells is preceded by autophagy—the movement of cell components into the vacuole or lysosomes with their subsequent degradation [23]. In wheat, both processes develop from the base of the ear to its apex, determining the thickness of the pericarp in grains of different levels of location. The importance of autophagy in the response of wheat seedlings to biotic and abiotic stress factors, usually discussed in connection with the antioxidant role of flavonoids, has been established [23]. The participation of the pericarp in seed development includes the synthesis and accumulation of its own products of photosynthesis and other compounds of primary and secondary metabolism, immobilization of catabolic products during programmed cell death, and transport of water and nutrients [20]. It is assumed that the pericarp mechanically limits the size of the caryopsis and its ability to fill grain. The contribution of photosynthesis to this process in various parts of the wheat ear, including the pericarp, increases significantly at the grain filling stage under conditions of water deficiency, which negatively affects the photosynthetic potential of the flag leaf [22]. A number of facts indicate specific features of photosynthesis occurring in the pericarp [21].

The testa (seed coat), which is a maternal tissue in origin, develops from the outer integument of the ovule. In mature grain, the pericarp and testa are represented by dead cells, including many accumulated compounds of secondary metabolism and catabolic products, as well as a number of enzymes that remain active in dead tissues [24]. These compounds include colored and uncolored flavonoids, which may promote seedling growth and persistence during early development.

The aleurone is the outer layer of the triploid endosperm, represented by one layer of cells in corn, wheat, and rye and three to four in rice and barley. The role of the endosperm is the accumulation and transport of nutrients. Modified aleurone cells are part of the conducting tissue located in the depths of the groove in Triticeae. This conductive system plays an important role in grain filling. Aleurone cells remain viable until seed germination and undergo programmed cell death after secretion of hydrolytic enzymes into the starchy endosperm and formation of a central vacuole. Destruction of the central vacuole coincides with the onset of programmed aleurone cell death [18].

The ability of uncolored and colored flavonoids (anthocyanins and proanthocyanidins) synthesized in grain hulls to absorb ultraviolet and broad-spectrum visible light can prevent the formation of reactive oxygen species (photoprotection) [1, 2]. The participation in stress reactions to light of trichomes, outgrowths of epidermal cells that form a pappus on the surface of the caryopsis facing the sun, is discussed. [25]. In rye grains, even with an uncolored pericarp, this section of the grain acquires transient anthocyanin color. There is evidence that chloroplasts that have completed their function are absorbed into the vacuole in pericarp cells (chlorophagy) as a result of a stress response to UV-B and intense visible light [23]. Trichomes in many plants contain anthocyanins. In *Arabidopsis*, MBW complexes formed by the bHLH-MYC transcription factors GL3 or EGL3 (GLABRA 3 or ENHANCER OF GLABRA 3) and the WD40 protein TTG1 (TRANSPARENT TESTA GLABRA 1) activate not only anthocyanin pigmentation of various organs but also the formation of trichomes [26–29]. This indicates a similar adaptive role of trichome formation and accumulation of flavonoid compounds. It should also be noted that there is pigment along the groove in wild-type rye grains. It is possible that the induced accumulation of anthocyanins in the structures of the caryopsis has an adaptive significance during grain filling under conditions of water stress and the accessibility of the pericarp to solar radiation in open-grain forms.

Anthocyanins and uncolored flavonoids are involved in the inactivation of excess reactive oxygen species. Colocalization of ROS and flavonoid sources is important for their antioxidant function [1, 2]. A number of species showlocalization of uncolored flavonoids not only in the vacuole but also in the nucleus, chloroplasts, and mitochondria. It is emphasized that the nuclear localization of flavonoids contributes not only to the "quenching" of ROS but also to the binding of transition metal ions, limiting the appearance of a new wave of ROS, and may also play a role in the regulation of gene activity [1]. However, the vast majority of uncolored and colored flavonoids in caryopsis tissues, as well as in the cells of vegetative tissues, accumulate in the vacuole. In cereals belonging to the Triticeaetribe, predominantly cyanidin

derivatives accumulate in the colored pericarp, delphinidin derivatives accumulate in the colored aleurone, and oligomeric and polymeric proanthocyanidins accumulate in the seed coat (testa). In the literature, seed coat proanthocyanidins are mainly discussed in connection with their adaptive role [30].

In wheat and barley, the seed coat contains proanthocyanidins formed as a result of the polymerization of flavan-3-ols (Fig. 1), mainly catechin [31]. Oxidized proanthocyanidins acquire a red-brown color. This color is characteristic of red grain wheat; in wild-type barley grain, proanthocyanidins are not oxidized and are colorless. In barley, they are present in high concentrations, giving sections of the seed coat a granular structure [32]. The testa is significantly thicker in wild-type barley than in mutants without proanthocyanidins. In red grain wheat, it was not possible to detect sequences orthologous to the gene *AtTT10* (*TRANSPARENT TESTA 10*), encoding laccase, an enzyme involved in the oxidation of proanthocyanidins in *Arabidopsis* [31]. In a mature rye grain, the seed coat is represented by a thin brown pigment layer. This layer gives a color reaction to reagents that detect proanthocyanidins [33]. In rice and millet grains, proanthocyanidins accumulate in the pericarp. In millet, in the pericarp, in addition to proanthocyanidins, similar polymers, phlobaphenes, can be synthesized from flavan-4-ols (Fig. 1) [34]. In corn, in addition to anthocyanins, exclusively phlobaphenes are formed in the pericarp. Proanthocyanidins and phlobaphenes are widely distributed in higher plants; in addition to fruits and seeds, they are found in the bark, leaves, and roots. The biological function of these polymers is unclear and may be species specific [34].

In wheat, as the grain develops, proanthocyanidin molecules lengthen, and only high-molecular-weight, non-extractable polymers remain in the mature grain. Their insolubility can also be explained by the formation of compounds with proteins and polysaccharides. In barley grain, in addition to insoluble ones, soluble oligomers from dimers to hexamers were also found [34]. In the breeding of malting barley, induced mutants with the absence or low content of proanthocyanidins are widely used, which, when bound to proteins, produce an undesirable suspension. Ten genes are known, mutations in which lead to the absence of proanthocyanidins in grain [32]. In cereals, the association of proanthocyanidins with premature germination (vivipary) and seed dormancy has long been discussed. Red grain wheat, unlike white grain wheat, is less prone to premature sprouting [31]. Barley, with its high content of proanthocyanidins, is characterized by a long period of post-harvest ripening. Rye is the most prone to premature germination.

In barley, mutations in the gene *Ant28* (*Anthocyaninless 28*), encoding the R2R3-MYB transcription factor, disrupt the biosynthesis of proanthocyanidins, but not anthocyanins. Gene *Ant28* is designated *HvMyb10* according to homology to gene *TaMyb10* in wheat [35]. In mutants for gene *HvAnt28/HvMyb10*, as expected, the seed dormancy period is reduced. However, the relationship between the accumulation of proanthocyanidins and the dormancy period is not confirmed for barley lines with a null allele in the flavanone-3-hydroxylase gene *Ant17*. Mutants for this gene do not synthesize proanthocyanidins in grain and anthocyanins in the plant and do not differ from the norm in terms of seed dormancy [36]. Unfortunately, the potential of a collection of barley mutants for 28 flavonoid biosynthesis genes [32] has not been fully exploited to identify the molecular function of these genes [37]. The mechanism of the possible action of proanthocyanidins in controlling seed dormancy is not clear. Reasons such as a physical obstacle to an increase in seed volume, a decrease in the permeability of the seed coat to water and/or oxygen, and the direct effect of proanthocyanidins and their precursors or metabolites on dormancy and seed germination are discussed [31]. It is well known that damage to the outer shells accelerates seed germination. From observations of the crops of wheat, barley, and rye, it follows that birds primarily visit wheat and barley; the rye grain remains untouched with this choice. The bitter taste of many fruits is attributed to proanthocyanidins and is considered a trait recognized by birds. The grain of all varieties of rye, unlike varieties of white wheat and malting barley, has a colored seed coat containing proanthocyanidins.

It is believed that the reduction in the dormancy period of seeds of cultivated plants occurred during domestication and subsequent selection [38]. Selection for shortening the dormant period could have led to a sharp decrease in genetic variability for this trait and premature germination of seeds of cultivated plants with excess moisture during the ripening period. Proanthocyanidins could have played a subordinate role in the variability of the dormant period. Feral rye, the ancestorof cultivated rye, has become a widespread weed in wheat crops in North Carolina [39]. The grain of this rye remains viable in the soil from one to several years. It is not known what role polymorphism in proanthocyanidin content plays in this variability. In rice, it has been shown that the main contribution to resistance to root sprouting is made by interactions of genes *SD6* (*Seed dormancy 6*) and *ICE2* (*Inducer of C-repeat binding factors expression 2*), encoding oppositely acting bHLH-MYC transcription factors. The effect of interaction between the alleles of these genes depends on temperature; it determines the content of abscisic acid in the embryo and the ability of the grains to germinate [40].

Using ten barley mutants for proanthocyanidin biosynthesis, it was shown that the wild type does not have greater resistance to Fusarium head blight compared to the mutants. On the contrary, high resistance was found in the mutant *ant18-159*, which accumulates a small amount of dihydroquercetin as a result of

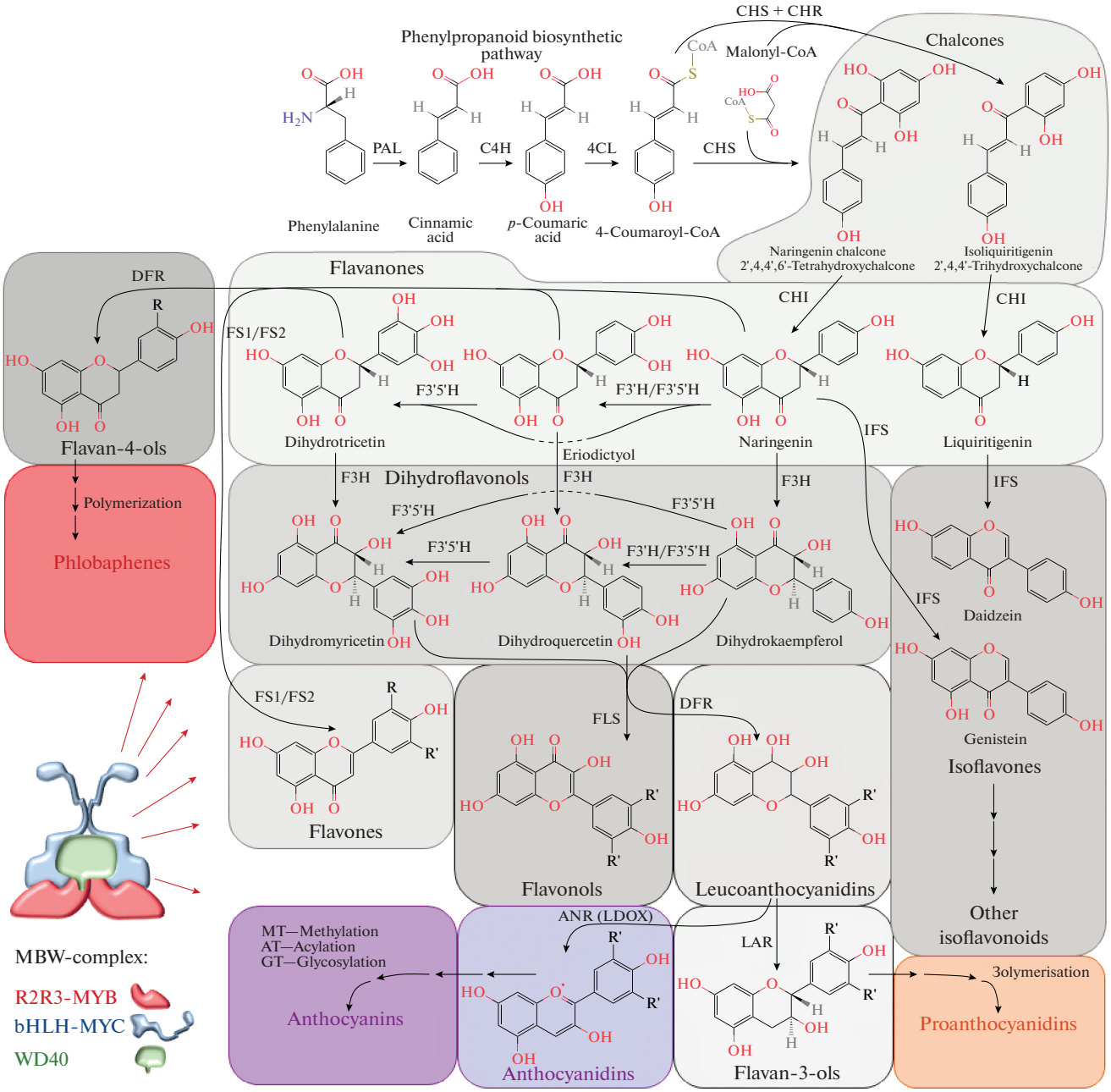

Fig. 1. Scheme of the flavonoid biosynthesis pathway in plants. Classes of uncolored flavonoids are highlighted in shades of gray, colored flavonoids (phlobaphenes, proanthocyanidins, anthocyanidins, and anthocyanins) are highlighted in colors. The biosynthesis pathway of flavonoids in plants begins, as with all phenolic compounds, with sequential transformations of phenylalanine catalyzed by phenylalanine ammonia-lyase (PAL), cinnamic acid 4-hydroxylase (C4H), and 4-coumarate-CoA ligase (4CL). Chalcone synthase (CHS) mediates the synthesis of naringenin chalcone, the precursor of most flavonoids, and the combined action of chalcone synthase and chalcone reductase (CHR) determines the synthesis of chalcone isoliquirintigenin, the precursor of isoflavonoids. Chalcones give rise to all classes of flavonoids, which is determined by the work of the following enzymes: chalcone isomerase (CHI), flavone synthase 1 and 2, isoflavone synthase (IFS), flavanone-3-hydroxylase (F3H), flavonoid3'- and 3',5'-hydroxylases (F3'H/F3'5'H), flavonol synthase (FLS), dihydroflavonol reductase (DFR), leucoanthocyanidin reductase (LAR), and anthocyanidin reductase/leucoanthocyanidin dioxygenase (ANR/LDOX). Anthocyanins are synthesized by modifying anthocyanidins through methyltransferases (MT), acyltransferases (AT), and glycosyltransferases (GT). Flavanones and dihydroflavonols can have different numbers of hydroxyl groups, which is mediated by the work of flavonoid 3'- and 3',5'-hydroxylases and subsequently leads to the synthesis of various phlobaphenes, flavones, flavonols, proanthocyanidins, and anthocyanidins. Thus, cyanidin is characterized by the presence of a hydroxyl group in the 3' position, and delphinidin, in both the 3' and 5' positions. The expression of various structural genes of flavonoid biosynthesis is directly activated by binding to their promoters of MBW complexes consisting of two dimerized bHLH-MYC transcription factor molecules, each associated with one R2R3-MYB transcription factor molecule. This complex is stabilized by the WD40 protein.

a nonsense mutation in the dihydroflavonol reductase gene [41].

The peculiarities of photosynthesis in a wheat ear may be associated with the development of seeds under stress conditions caused by lack of moisture and excess light, which accompanies grain filling in cereals. [19, 21]. Unfavorable conditions during the formation of grains negatively affect the fertility and survival of seedlings. In the wild ancestors of modern cereals, the appearance of complex antioxidant protection in the fruit, seed coats, and aleurone could have been associated with ensuring the survival of the embryo and seedlings under unfavorable conditions of development. This system can participate in antistress reactions during seed germination under conditions of low temperatures in winter cereals and lack of moisture in spring forms. It can be assumed that in northern cereals the formation of specific anthocyanin metabolism in the aleurone was influenced by selection under conditions of high-mountain settlement. For its formation, it turned out that a mutational change in only one regulatory gene, as in the case of black rice, was not enough. The selection affected the amino acid sequence of the key enzyme of delphinidin biosynthesis—flavonoid 3',5'-hydroxylase F3'5'H—and the regulatory sequences of the genes of two transcription factors, R2R3-MYB and bHLH-MYC, and ultimately led to the cluster organization of three genes that control biosynthesis of delphinidin in the aleurone in some representatives of species of the subfamily Pooideae, which will be discussed in the next section.

ORIGIN AND EVOLUTION OF GRAINCOLOR GENES IN CULTIVATED CEREALS

The origin and evolution of flavonoid biosynthesis is associated with the emergence of plants on land [42]. The variety of contrasting conditions of the new environment was a factor in the selection of adaptive mutations. Such mutations could have occurred in genes that control primary metabolism and are represented in the genome by several copies resulting from duplication of individual sequences or the entire genome. Phylogenetic analysis showed that the genes of the two first enzymes of flavonoid biosynthesis, chalcone synthase (CHS) and chalcone isomerase (CHI), originated from genes involved in fatty acid metabolism. Such evolutionary connections can be traced for all structural and regulatory genes that control not only the biosynthesis of flavonoids but also secondary metabolism in general [42]. Features of the molecular structure of flavonoids determined their participation in the adaptive evolution of all groups of land plants on the basis of the general mechanisms of their action [42]. The biosynthesis of flavonoids is regulated by a complex of transcription factors, universal for plants, integrated into the general system of metabolic regulation. During the evolution of plants, at the intraspecific and interspecific levels, various variants of this complex developed, regulated ontogenetically and in response to changing environmental conditions.

The material for evolutionary transformations in cultivated plants is spontaneous genetic variability, which supplies material for domestication and selection. Obviously, selection for traits useful to humans did not always lead to an improvement in the adaptive properties of plants. This discrepancy arises owing to both pleiotropy and the linkage effect of genes with different functions. The color of the caryopsis is not considered a characteristic of the domestication syndrome [43]. Wild ancestors and cultivated plants differ in the frequency of occurrence of active and inactive alleles of genes that control the biosynthesis of flavonoids in individual caryopsis membranes. The adaptive role of the color of the grain (dry fruit) has not been strictly proven; it is referred to by analogy with the color of the generative organs in dicotyledonous plants [10] or is considered obvious on the basis of general ideas about the function of anthocyanins [1, 9]. When discussing the adaptive role of anthocyanin coloration in grains, or more precisely the role of colored flavonoids, one must take into account their evolutionary origin and the existing morphophysiological and ecological differences between species. Such differences have developed between grains of southern origin (corn, rice, millet) and northern cereals. Wheat, barley, and rye have spring and winter forms; northern cereals are frost-resistant and moisture-loving, require long days for development, and are characterized by a whole complex of distinctive features that can be associated with the biosynthesis of flavonoids in the tissues of the grain and vegetative organs.

The adaptive role of flavonoids is established as a change in their concentration or transcriptional activity of their biosynthesis genes in response to a stress factor [5]. This approach can be called ecophysiological. Another approach is based on phylogenetic analysis of genes and modeling the structure and function of proteins encoded by these genes. The established differences between paralogs and/or orthologs are explained by the action of selection factors of a natural or artificial nature.

Evolution of Structural Genes for Flavonoid Biosynthesis

Phenolic compounds in plants are synthesized predominantly from phenylalanine through the phenylpropanoid biosynthesis pathway, one of the branches of which is the biosynthesis of various classes of flavonoids (Fig. 1) [9]. A common and key reaction for all classes of flavonoids is the synthesis of chalcones by chalcone synthase (CHS) and their isomerization by chalcone-flavanone isomerase (CHI) to form flavanones. The work of further various enzymes causes successive transformations with the formation of many classes of flavonoids, including flavonols, proantho-

cyanidins, and anthocyanidins (Fig. 1). The molecules of these compounds undergo additional modifications with sugars, methyl groups, and other substituents with the participation of many additional enzymes, which gives them various properties that both are important for plant adaptation and bring a benefit to humans [9, 44, 45].

In cereals, phylogenetic analysis was carried out for structural genes encoding flavonoid 3'-hydroxylase (F3'H) [46] and flavonoid 3',5'-hydroxylase (F3'5'H) [47]. These enzymes are involved in the B-ring hydroxylation of flavanones and dihydroflavonols during the initial stages of the biosynthesis of the anthocyanidins cyanidin and delphinidin, respectively. Two independent lines of evolution of flavonoid 3'-hydroxylase genes have been identified, presumably arising after duplication of the original sequence in the common ancestor of monocots and subsequent doubling, and then loss of a number of copies in individual taxa [46]. It is assumed that the detected functional differences between the enzymes encoded by genes from different phylogenetic lineages are caused by positive natural selection influencing substrate binding by the active site. According to the authors, this may explain the new 5'-hydroxylase activity of enzymes from one of the evolutionary lines in rice and other cereals in relation to chrysoeriol. As a result of this reaction, the flavonoids selgin and then tricine, which performs important protective and structural functions in cereals, are formed [46]. Species-specific transcriptional differences were found between paralogs encoding F3'H. In barley, one gene is transcribed in the aleurone, pericarp, and lemma/palea, and its copy is transcribed in the vegetative organs [48]. One of the flavonoid 3',5'-hydroxylase genes is active in the aleurone, and its copy is transcribed in many tissues [48].

Flavonoid 3',5'-hydroxylases in cereals can also be divided into two classes, named Mo_F35H1 and Mo_F35H2 (Monocot F3'5'H) [47]. It is assumed that genes for enzymes of different classes arose through duplication in the genome of a common ancestor and further divergence during speciation under the influence of selection [47]. Sequences encoding Mo_F35H1 are found in all studied species of the family Poaceae, and sequences Mo_F35H2 are found only in some species of the subfamily Pooideae, which include wheat, barley, and rye. Mo_F35H2 proteins are more similar to flavonoid 3',5'-hydroxylases of dicotyledonous plants than to Mo_F35H1. According to the authors, this similarity indicates the convergent adaptive evolution of flavonoid 3',5'-hydroxylases specific to Pooideae and flavonoid 3',5'-hydroxylases of dicotyledons at the level of amino acid substitutions [47].

The chalcone isomerase (CHI) genes were also subject to specific evolution in Triticeae [49]. However, in this case, evolution did not affect the protein-coding sequence, but the exon-intron structure of genes *Chi*. It was shown that, in the putative ancestor of cereals, the chalcone isomerase gene contained four exons and three introns. This structure has been preserved to this day in most cereals, including corn and rice; however, all representatives of the Triticeae tribe are characterized by the loss of the third intron. In the rye *Secale cereale*, the second intron is additionally lost, as a result of which its chalcone isomerase gene contains only two exons. The structure of the CHI protein is conservative and has no fundamental differences in all cereals [49]. What causes the loss of introns in a gene *Chi* in Triticeae is unknown; however, this phenomenon is also observed in other groups of plants [50], as well as in some other gene families [51, 52]. It is assumed that deletion of introns may contribute to faster transcription of the gene, which in the case of chalcone isomerase may indicate adaptation to unfavorable conditions through the rapid accumulation of flavonoid compounds [49, 53].

Evolution of Regulatory Genes for Flavonoid Biosynthesis

The key regulators of the temporal and spatial specificity of flavonoid biosynthesis are proteins of the bHLH-MYC, R2R3-MYB, and WD40 families, which form MBW complexes (Fig. 1) [10]. Since the first data on the joint regulation of flavonoid biosynthesis by these proteins obtained from the study of regulatory genes *R* (*Red*), *C1* (*Colorless 1*), and *PAC1* (*Pale aleurone color 1*) in maize [54–60] and genes *GL1*, *GL3* (*GLABRA*), *TT2*, *TT8* (*TRANSPARENT TESTA*), and *TTG1* (*TRANSPARENT TESTA GLABRA 1*) in *Arabidopsis* [61–65], MBW complexes that regulate the biosynthesis of flavonoids and, in particular, anthocyanins were identified in many plants. The diversity of MYC and MYB genes determines the specificity and intensity of flavonoid biosynthesis and can form complex variants of plant pigmentation [59, 63, 65–73].

Barley structural gene *HvF35H* (*HvF3'5'H*) colocalizes together with two regulatory genes *HvMYB4H* (*HvMpc2*) and *HvMYC4H* (*HvMyc2*) in the trigenic cluster MbHF35 (MYB-bHLH-F3'5'H) localized on chromosome 4H [47, 74]. All three genes are specifically expressed in the aleurone. The cluster organization of delphinidin biosynthesis genes in aleurone, in addition to barley, has been established for two more cultivated cereals—wheat with a cluster localized on chromosome 4D and rye with a cluster in a homeologous fragment of chromosome 7R. In *Aegilops tauschii*, the donor of D genomeof bread wheat, the cluster organization is retained, but the homolog *HvMYC4H* in *Aegilops tauschii* is represented by a nonfunctional allele or pseudogene, which corresponds to the absence of aleurone coloration in the studied forms of this species [47]. The genes included in the cluster constitute distinct phylogenetic lineages in Triticeae species. Localization in homeologous chromosome fragments in wheat and *Aegilops* (4D), barley (4H),

and rye (7R) indicates the origin of the cluster from a common ancestor and the parallel evolution of genes providing the biosynthesis of delphinidin derivatives in the aleurone. It was established that the sequence of these genes evolved under the influence of natural selection. Thirteen amino acid residues have been identified in the structure of the HvF35H protein, which could have appeared as a result of natural selection for the thermal stability of the protein molecule [46, 47]. Cereal grains are exposed to intense light and heat during the critical period of grain filling. "Blue" anthocyanins have a wider absorption spectrum and are better photoprotectors and antioxidants than "red" anthocyanins [47]. These differences could have contributed to the formation of an effective defense system based on delphinidin derivatives in the ancestral species of barley, wheat, and rye which occupied high-mountain areas with sharp fluctuations in daytime temperature, intense light, and moisture deficiency. It is likely that the close linkage of genes that control delphinidin biosynthesis is necessary for the joint regulation of their transcription and accelerated response to stress factors.

All studied forms of wild barley *H. spontaneum* are fixed by active aleurone color alleles in regulatory genes *HvMYB4H* and *HvMYC4H*. Most of them have blue grain. Local blue grain varieties *H. vulgare* also carry active aleurone color alleles in the genes *HvMYB4H* and *HvMYC4H*. In white grain barley varieties, one or both genes are represented by inactive alleles. It is most likely that the predominance of these alleles in cultivated barley is associated with its domestication and subsequent selection of white grain forms [47].

Blue aleurone trait in common wheat is controlled by loci included in the genome of individual varieties during distant hybridization. Blue grain loci *Ba1* and *Ba2* (*Blue aleurone*) are established in diploid Triticeae species *Thinopyrum ponticum* (syn. *Agropyron elongatum*) and einkorn wheat *Ttiticum monococcum*, the cultivated form *T. boeoticum*, respectively [75]. Gene *ThMYC4E* (locus *Ba1*) as part of the whole chromosome 4E or its fragments ensures the synthesis of anthocyanins in aleurone in wheat obtained on the basis of hybridization with *Thinopyrum ponticum* [76]. Orthologs of three barley genes *HvMYC4H, HvMYB4H*, and *Hv35H* have been found in blue-grained wheat. These genes are part of the locus *Ba1* designated as *TaMYC4D*, *TaMYB4D*, and *TaF35H*, respectively [46]. Active gene markers *ThMYC4E* are not found in the studied samples of *T. urartu, T. monococcum*, *T. turgidum*, *Aegilops tauschii*, and *T. aestivum* with uncolored aleurone [76]. Diploid wheat gene *T. monococcum TbMYC4A* (locus *Ba2*) is an ortholog of genes *ThMYC4E* and *HvMyc2/HvMYC4H*. An allele-specific marker of this gene has not been found in white grain breeds *T. urartu*, *Ae. tauschii*, *T. turgidum*, *T. araraticum*, and *T. zhukovskyi* [77]. In rye, the localization of the genes that make up the three-gene cluster was carried out on the basis of the similarity of the mapped contigs [78] with sequenced barley genes [7, 8]. Obviously, the conclusion about the presence of a functional cluster of genes in rye that controls the biosynthesis of delphinidin in the aleurone requires experimental confirmation.

The vast majority of bread wheat and barley varieties are white grain forms that do not contain anthocyanins in the aleurone and, therefore, carry inactive alleles in at least one of the aleurone color genes or genes responsible for anthocyanin biosynthesis throughout the plant. The predominance of uncolored genotypes in wheat and barley is historically associated with technological requirements for processing grain into bread and beer. Flour yield and whiteness in wheat and low proanthocyanidin content in barley malt were among the main traits targeted for selection in baking wheat and malting barley, respectively. In rye, owing to its allogamous nature, intravarietal polymorphism in aleurone color is observed. It remains unclear in what combinations the active and inactive alleles of these genes are present in modern wheat, barley, and rye, as well as in related wild species of Triticeae.

The role of anthocyanin grain color (the presence of colored flavonoids) in cultivated plants and their wild ancestors, as an adaptive trait, remains unclear. Most modern varieties of cereals do not have anthocyanin coloration in their grains. Its absence is associated with the fixation of inactive alleles of regulatory genes, which received preference during the domestication and selection of corn, rice, millet, wheat, and barley [6]. During the evolution and selection of corn, a reversion occurred from the uncolored grain of the ancestral teosinte species through the variety of aleurone colors in primitive South American forms of corn to the uncolored grain of modern open-pollinated varieties and hybrid varieties. Owing to the high activity of transposons in local forms, dominant mutations of the regulatory genes *C1* (*Colorless 1*) of the R2R3-MYB transcription factor and *R1* (*Red 1*) of the bHLH-MYC transcription factor arose and, probably owing to aesthetic preference, became established, leading to the accumulation of anthocyanins in the aleurone and the appearance of corn with black, blue, and red grains [79]. With further adaptation and selection of corn in Europe and America, the number of colored forms decreased, and after the introduction of hybrid varieties, yellow corn became dominant [80]. When creating inbred lines and hybrids, preference was given to more productive forms of corn with uncolored grain. The red color of the grain is characteristic of wild rice *Oryza rufipogon*, which has a common ancestor with cultivated white grain rice. Differences in color are controlled by two genes: the gene *Rc*, which encodes the bHLH transcription factor, and the gene *Rd*, encoding dihydroflavonol-4-reductase. These genes are involved in the biosynthesis of proanthocyanidins, the oxidation of which results in the formation of red pigment in the pericarp. Recessive

mutations in the gene *Rc* lead to a lack of pigment; one of these mutations became widespread during the domestication of rice [81]. Most bread wheat varieties are uncolored or have a red grain color owing to the presence of proanthocyanidins in the seed coat. Their biosynthesis is controlled by homeologous MYB transcription factor genes *TaR-1* (*Red-1*): *Tamyb10-A1* at 3A, *Tamyb10-B1* on 3B, and *Tamyb10-D1* on 3D wheat chromosomes [82, 83]. These wheat genes are orthologs of the gene *HvANT28/Hvmyb10* of barley and are evolutionarily close to the gene *AtTT2* of *Arabidopsis*, each of which also positively regulates proanthocyanidin biosynthesis [35, 82, 83].

The rarest among cultivated cereals are varieties with colored pericarp. Black (purple) grain color has not been described in wild rice; it is found only in local varieties of cultivated rice and appeared during or after the domestication of rice in Ancient China. The origin of black rice is associated with rearrangements in the promoter region of the plant color gene *OsB2* (*Booster 2/Os04g0557500*), encoding the bHLH transcription factor. Ectopic expression in the pericarp of a mutant gene (alleles of the gene *OsB2*), named *Kala4* [84], leads to anthocyanin biosynthesis in the presence of a dominant allele of the gene *Kala3*, encoding the R2R3-MYB transcription factor. This allele in rice with anthocyanin in the pericarp includes two tandem repeats in the promoter instead of one in uncolored rice [85]. During the domestication of grain amaranth, the anthocyanin coloring of the grain was lost. Moreover, this happened three times on the basis of one wild ancestral species, and in all cases, the cultivated species included mutations that independently arose in the locus of the gene of the transcription factor MYB—the ortholog of the gene *C1* of corn [86].

The purple color of the pericarp in hexaploid wheat is associated with the introgression of fragments of chromosomes 2A and 7D from violet-grain tetraploid wheat from Ethiopia. Dominant complementary genes *Pp3* (*Purple pericarp 3*, chromosome 2A) and *Pp1* (*Purple pericarp 1*, chromosome 7D) have been identified in hexaploid wheat with pericarp coloration [87–89]. Gene *Pp3/TaPpb1* encodes the bHLH-MYC transcription factor, and the gene *Pp1/TaPpm1* encodes the MYB transcription factor [90]. It has been shown that the allele of gene *Pp3/TaPpb1/TaMYC1* in white wheat in the proximal region of the promoter contains one copy of a sequence of 261 nucleotides, and in wheat with pericarp coloring, it contains six copies organized in the form of tandem repeats [90, 91]. In addition, in white grain wheat, three nonfunctional allelic variants were found in the coding sequence of this gene [90].

The vast majority of barley varieties have yellow grains. Red and purple grain colors have been found in some forms of wild barley *Hordeum spontaneum* and certain forms of cultivated barley. It is controlled by a dominant allele *Pre2*, which is presumably one of the alleles of the gene for anthocyanin coloring of vegetative parts of the plant *Ant2*, encoding the bHLH-MYC transcription factor, in combination with the MYB factor Ant1, activating anthocyanin biosynthesis [92–94].

Numerous forms of rye *Secale cereale* described within 42 subspecies have anthocyanin coloring of individual organs varying in intensity: coleoptile, leaves, nodes, internodes, and ear elements. Each of them has polymorphism in grain color [95]. The vast majority of plants *Secale cereale* have grains of yellow and green color, much less often brown, and very rarely red or purple color. Among the studied forms of *Secale cereale* subsp. *cereale*, red and purple coloring of the grain is described only in individual samples of weedy rye. Monohybrid segregation based on the purple color of rye grain was established by different authors using independently obtained material [96]. There is reason to believe that all purple grain forms found in rye carry dominant alleles of the same purple grain gene *Vs* (*Violet seed*). Gene *Vs* by the authors of the present publication [96] was transferred to the spring autofertile background. Using this form and translocation analysis, gene *Vs* was localized on chromosome 2R under the symbol *Ps* (*Purple seed*) [97]. We have obtained data indicating the orthology of the gene *Vs/Ps* and genes of transcription factors bHLH-MYC in rice, wheat, and barley that control the biosynthesis of anthocyanins in the pericarp of the caryopsis. It is most likely that in rye the presence of anthocyanins in the pericarp is under the control of the MBW complex, which includes factors of the transcription of the families R2R3-MYB and bHLH-MYC and the WD40 protein.

CONCLUSIONS

The role of flavonoids in cereal grains may include photoprotection and neutralization of reactive oxygen species formed during photosynthesis in the pericarp, as well as their protective effect during seed germination. During swelling and germination of seeds, activation of respiration in the aleurone leads to the accumulation of reactive oxygen species, which play a regulatory role and, when in excess, have a toxic effect. The restoration of the redox balance is facilitated by both flavonoids and antioxidant enzymes, which retain their activity in the dead tissues of the grain [98]. It has been established that hundreds of proteins in grain shells and glumes can remain active for decades, being released into the environment during seed germination. These enzymes, along with antioxidants accumulated in the shells, contribute to the survival and active growth of seedlings [24]. The tissue-specific biosynthesis of delphinidin in the aleurone and proanthocyanidins in the seed coat can be considered a more universal adaptation than the biosynthesis of cyanidin derivatives in the pericarp. The color of the pericarp was found in a limited number of wild species of cereal plants and did not become widespread during

domestication and selection. The color of the pericarp can be attributed to local adaptation [99], characteristic of individual populations and having simple genetic control. It may be based on dominant mutational changes (gain-of-function mutations) in the regulatory elements of the genes of transcription factors bHLH-MYC and R2R3-MYB, leading to ectopic expression of these genes. However, this conclusion is not supported by data obtained in field studies combining analysis of the frequency of pericarp coloration and geological, geographical, and meteorological characteristics of habitats with analysis of functional markers of genes of bHLH-MYC. Currently, the biological activity of flavonoids is much better studied from a dietary and medical point of view than in relation to their importance for plants themselves. The focus is on creating varieties of cereals—corn, rice, wheat, and barley—with high anthocyanin content [100, 101]. Genetic and biotechnological research is also aimed at creating plant forms with a high content of intermediate compounds of anthocyanin biosynthesis [102] or obtaining genotypes combining high content of anthocyanins and other compounds of secondary metabolism [103]. In this regard, rye is of particular interest with its characteristics of secondary metabolism, largely related to cross-pollination and the secondary nature of domestication, which proceeded like the evolution of a weedy plant in barley and wheat crops. The self-incompatibility of rye ensures a high level of heterogeneity in open-pollinated rye populations, including in genes that control secondary metabolism. The preserved and widespread weedy rye makes it possible to use its population as additional material for solving the evolutionary-genetic problems of secondary metabolism in cultivated cereals. The sequencing of the genomes of two rye samples [7, 8] opens up new opportunities for such research.

FUNDING

This study was carried out with partial support from the World-class Scientific Center " Agrotechnologies for the Future", in accordance with agreement No. 075-15-2022-322 dated April 22, 2022.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

REFERENCES

1. Agati, G., Brunetti, C., Fini, A., et al., Are flavonoids effective antioxidants in plants? Twenty years of our investigation, *Antioxidants*, 2020, vol. 9, no. 11, p. 1098. https://doi.org/10.3390/antiox9111098
2. Sharma, P., Jha, A.B., Dubey, R.S., and Pessarakli, M., Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions, *J. Bot.*, 2012, vol. 2012, p. 681. https://doi.org/10.1155/2012/217037
3. Coe, E.H., Neuffer, M.G., and Hoisington, D.A., The genetics of corn, in *Corn and Corn Improvement*, Madison: American Society of Agronomy, 1988, pp. 81–259. https://doi.org/10.2134/agronmonogr18.3ed.c3
4. Huang, H., Ullah, F., Zhou, D.X., et al., Mechanisms of ROS regulation of plant development and stress responses, *Front. Plant Sci.*, 2019, vol. 10, pp. 440–478. https://doi.org/10.3389/fpls.2019.00800
5. Yan, W., Li, J., Lin, X., et al., Changes in plant anthocyanin levels in response to abiotic stresses: a meta-analysis, *Plant Biotechnol. Rep.*, 2022, vol. 16, no. 5, pp. 497–508. https://doi.org/10.1007/S11816-022-00777-7
6. Paauw, M., Koes, R., and Quattrocchio, F.M., Alteration of flavonoid pigmentation patterns during domestication of food crops, *J. Exp. Bot.*, 2019, vol. 70, no. 15, pp. 3719–3735. https://doi.org/10.1093/jxb/erz141
7. Li, G., Wang, L., Yang, J., et al., A high-quality genome assembly highlights rye genomic characteristics and agronomically important genes, *Nat. Genet.*, 2021, vol. 53, no. 4, p. 574. https://doi.org/10.1038/S41588-021-00808-Z
8. Rabanus-Wallace, M.T., Hackauf, B., Mascher, M., et al., Chromosome-scale genome assembly provides insights into rye biology, evolution and agronomic potential, *Nat. Genet.*, 2021, vol. 53, no. 4, pp. 564–573. https://doi.org/10.1038/s41588-021-00807-0
9. Winkel-Shirley, B., Flavonoid biosynthesis: a colorful model for genetics, biochemistry, cell biology, and biotechnology, *Plant Physiol.*, 2001, vol. 126, pp. 485–493. https://doi.org/10.1104/pp.126.2.485
10. Davies, K.M., Albert, N.W., and Schwinn, K.E., From landing lights to mimicry: the molecular regulation of flower colouration and mechanisms for pigmentation patterning, *Funct. Plant Biol.*, 2012, vol. 39, no. 8, pp. 619–638. https://doi.org/10.1071/FP12195
11. Pucker, B. and Selmar, D., Biochemistry and molecular basis of intracellular flavonoid transport in plants, *Plants*, 2022, vol. 11, no. 7, p. 963. https://doi.org/10.3390/plants11070963/S1
12. Buhrman, K., Aravena-Calvo, J., Ross Zaulich, C., et al., Anthocyanic vacuolar inclusions: from biosynthesis to storage and possible applications, *Front. Chem.*, 2022, vol. 10, p. 913324. https://doi.org/10.3389/fchem.2022.913324
13. Petrussa, E., Braidot, E., Zancani, M., et al., Plant flavonoids-biosynthesis, transport and involvement in

stress responses, *Int. J. Mol. Sci.*, 2013, vol. 14, no. 7, pp. 14950–14973.
https://doi.org/10.3390/ijms140714950

14. Mackon, E., Jeazet Dongho, Epse Mackon, G.C., Ma, Y., et al., Recent insights into anthocyanin pigmentation, synthesis, trafficking, and regulatory mechanisms in rice (*Oryza sativa* L.) caryopsis, *Biomolecules*, 2021, vol. 11, no. 3, p. 394.
https://doi.org/10.3390/biom11030394
15. Zhu, Q., Xie, X., Xiang, G., et al., *In silico* analysis of a MRP transporter gene reveals its possible role in anthocyanins or flavonoids transport in *Oryza sativa, Am. J. Plant Sci.*, 2013, vol. 4, pp. 555–560.
https://doi.org/10.4236/ajps.2013.43072
16. Li, T., Zhang, W., Yang, H., et al., Comparative transcriptome analysis reveals differentially expressed genes related to the tissue-specific accumulation of anthocyanins in pericarp and aleurone layer for maize, *Sci. Rep.*, 2019, vol. 9, no. 1, p. 6023.
https://doi.org/10.1038/s41598-018-37697-y
17. Tan, X., Li, K., Wang, Z., et al., A review of plant vacuoles: formation, located proteins, and functions, *Plants*, 2019, vol. 8, no. 9, p. 327.
https://doi.org/10.3390/plants8090327
18. Zheng, Y., Zhang, H., Deng, X., et al., The relationship between vacuolation and initiation of PCD in rice (*Oryza sativa*) aleurone cells, *Sci. Rep.*, 2017, vol. 7, no. 1, p. 41245.
https://doi.org/10.1038/srep41245
19. Henry, R.J., Furtado, A., and Rangan, P., Pathways of photosynthesis in non-leaf tissues, *Biology*, 2020, vol. 9, no. 12, p. 438.
https://doi.org/10.3390/biology9120438
20. Legland, D., Le, T.D.Q., Alvarado, C., et al., New growth-related features of wheat grain pericarp revealed by synchrotron-based X-ray micro-tomography and 3D reconstruction, *Plants*, 2023, vol. 12, no. 5, p. 1038.
https://doi.org/10.3390/plants12051038
21. Simkin, A.J., Faralli, M., Ramamoorthy, S., Lawson, T., et al., Photosynthesis in non-foliar tissues: implications for yield, *Plant J.*, 2020, vol. 101, no. 4, pp. 1001–1015.
https://doi.org/10.1111/tpj.14633
22. Kong, L.A., Xie, Y., Sun, M.Z., et al., Comparison of the photosynthetic characteristics in the pericarp and flag leaves during wheat (*Triticum aestivum* L.) caryopsis development, *Photosynthetica*, 2016, vol. 54, no. 1, pp. 40–46.
https://doi.org/10.1007/S11099-015-0153-y
23. Li, Y.B., Yan, M., Cui, D.Z., et al., Programmed degradation of pericarp cells in wheat grains depends on autophagy, *Front. Genet.*, 2021, vol. 12, p. 784545.
https://doi.org/10.3389/fgene.2021.784545
24. Grafi, G. and Singiri, J.R., Cereal husks: versatile roles in grain quality and seedling performance, *Agronomy*, 2022, vol. 12, no. 1, p. 172.
https://doi.org/10.3390/agronomy12010172
25. Karabourniotis, G., Liakopoulos, G., Nikolopoulos, D., and Bresta, P., Protective and defensive roles of non-glandular trichomes against multiple stresses: structure–function coordination, *J. For. Res.*, 2020, vol. 31, no. 1, pp. 1–12.
https://doi.org/10.1007/s11676-019-01034-4
26. Walker, A.R., Davison, P.A., Bolognesi-Winfield, A.C., et al., The *TRANSPARENT TESTA GLABRA1* locus, which regulates trichome differentiation and anthocyanin biosynthesis in *Arabidopsis*, encodes a WD40 repeat protein, *Plant Cell*, 1999, vol. 11, no. 7, pp. 1337–1349.
https://doi.org/10.1105/tpc.11.7.1337
27. Morohashi, K. and Grotewold, E., A systems approach reveals regulatory circuitry for *Arabidopsis* trichome initiation by the GL3 and GL1 selectors, *PLoS Genet.*, 2009, vol. 5, no. 2, p. e1000396.
https://doi.org/10.1371/journal.pgen.1000396
28. Morohashi, K., Zhao, M., Yang, M., et al., Participation of the *Arabidopsis* bHLH factor GL3 in trichome initiation regulatory events, *Plant Physiol.*, 2007, vol. 145, no. 3, pp. 736–746.
https://doi.org/10.1104/pp.107.104521
29. Pesch, M., Schultheiß, I., Klopffleisch, K., et al., TRANSPARENT TESTA GLABRA1 and GLABRA1 compete for binding to GLABRA3 in *Arabidopsis, Plant Physiol.*, 2015, vol. 168, no. 2, pp. 584–597.
https://doi.org/10.1104/pp.15.00328
30. Khlestkina, E., The adaptive role of flavonoids: emphasis on cereals, *Cereal Res. Commun.*, 2013, vol. 41, no. 2, pp. 185–198.
https://doi.org/10.1556/crc.2013.0004
31. Vaughan, S.P., Baker, J.M., Primavesi, L.F., et al., Proanthocyanidin biosynthesis in the developing wheat seed coat investigated by chemical and RNA-Seq analysis, *Plant Direct.*, 2022, vol. 6, no. 10, p. e453.
https://doi.org/10.1002/pld3.453
32. Jende-Strid, B., Genetic control of flavonoid biosynthesis in barley, *Hereditas*, 1993, vol. 119, no. 2, pp. 187–204.
https://doi.org/10.1111/j.1601-5223.1993.00187.x
33. Zykin, P.A., Andreeva, E.A., Lykholay, A.N., et al., Anthocyanin composition and content in rye plants with different grain color, *Molecules*, 2018, vol. 23, no. 4, p. 948.
https://doi.org/10.3390/molecules23040948
34. Dixon, R.A. and Sarnala, S., Proanthocyanidin biosynthesis–a matter of protection, *Plant Physiol.*, 2020, vol. 184, no. 2, pp. 579–591.
https://doi.org/10.1104/pp.20.00973
35. Himi, E., Yamashita, Y., Haruyama, N., et al., *Ant28* gene for proanthocyanidin synthesis encoding the R2R3-MYB domain protein (Hvmyb10) highly affects grain dormancy in barley, *Euphytica*, 2012, vol. 188, no. 1, pp. 141–151.
https://doi.org/10.1007/S10681-011-0552-5
36. Himi, E. and Taketa, S., Barley Ant17, encoding flavanone 3-hydroxylase (F3H), is a promising target locus for attaining anthocyanin/proanthocyanidin-free plants without pleiotropic reduction of grain dormancy, *Genome*, 2015, vol. 58, no. 1, pp. 43–53.
https://doi.org/10.1139/gen-2014-0189
37. Shoeva, O.Yu., Strygina, K.V., and Khlestkina, E.K., Genes determining the synthesis of flavonoid and melanin pigments in barley, *Vavilovskii Zh. Genet. Sel.*,

2018, vol. 22, no. 3, pp. 333–342.
https://doi.org/10.18699/VJ18.369

38. Flint-Garcia, S.A., Genetics and consequences of crop domestication, *J. Agric. Food Chem.*, 2013, vol. 61, no. 35, pp. 8267–8276.
https://doi.org/10.1021/jf305511d
39. Sun, M. and Corke, H., Population genetics of colonizing success of weedy rye in Northern California, *Theor. Appl. Genet.*, 1992, vol. 83, no. 3, pp. 321–329.
https://doi.org/10.1007/BF00224278
40. Xu, F., Tang, J., Wang, S., et al., Antagonistic control of seed dormancy in rice by two bHLH transcription factors, *Nat. Genet.*, 2022, vol. 54, no. 12, pp. 1972–1982.
https://doi.org/10.1038/s41588-022-01240-7
41. Skadhauge, B., Thomsen, K.K., and von Wettstein, D., The role of the barley testa layer and its flavonoid content in resistance to *Fusarium* infections, *Hereditas*, 1997, vol. 126, no. 2, pp. 147–160.
https://doi.org/10.1111/J.1601-5223.1997.00147.X
42. Yonekura-Sakakibara, K., Higashi, Y., and Nakabayashi, R., The origin and evolution of plant flavonoid metabolism, *Front. Plant Sci.*, 2019, vol. 10, p. 943.
https://doi.org/10.3389/fpls.2019.00943
43. Pankin, A. and von Korff, M., Co-evolution of methods and thoughts in cereal domestication studies: a tale of barley (*Hordeum vulgare*), *Curr. Opin. Plant Biol.*, 2017, vol. 36, pp. 15–21.
https://doi.org/10.1016/j.pbi.2016.12.001
44. Alappat, B. and Alappat, J., Anthocyanin pigments: beyond aesthetics, *Molecules*, 2020, vol. 25, no. 23, p. 5500.
https://doi.org/10.3390/molecules25235500
45. Khoo, H.E., Azlan, A., Tang, S.T., and Lim, S.M., Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits, *Food Nutr. Res.*, 2017, vol. 61, no. 1, p. 1361779.
https://doi.org/10.1080/16546628.2017.1361779
46. Jia, Y., Li, B., Zhang, Y., et al., Evolutionary dynamic analyses on monocot flavonoid 3'-hydroxylase gene family reveal evidence of plant-environment interaction, *BMC Plant Biol.*, 2019, vol. 19, no. 1, p. 347.
https://doi.org/10.1186/s12870-019-1947-z
47. Jia, Y., Selva, C., Zhang, Y., et al., Uncovering the evolutionary origin of blue anthocyanins in cereal grains, *Plant J.*, 2020, vol. 101, no. 5, pp. 1057–1074.
https://doi.org/10.1111/tpj.14557
48. Vikhorev, A.V., Strygina, K.V., and Khlestkina, E.K., Duplicated flavonoid 3'-hydroxylase and flavonoid 3', 5'-hydroxylase genes in barley genome, *Peer J.*, 2019, vol. 2019, no. 1, p. e6266.
https://doi.org/10.7717/peerj.6266
49. Khlestkina, E.K. and Shoeva, O.Y., Intron loss in the chalcone-flavanone isomerase gene of rye, *Mol. Breed.*, 2014, vol. 33, no. 4, pp. 953–959.
https://doi.org/10.1007/s11032-013-0009-8
50. van Tunen, A.J., Koes, R.E., Spelt, C.E., et al., Cloning of the two chalcone flavanone isomerase genes from *Petunia hybrida*: coordinate, light-regulated and differential expression of flavonoid genes, *EMBO J.*, 1988, vol. 7, no. 5, pp. 1257–1263.
https://doi.org/10.1002/j.1460-2075.1988.tb02939.x
51. Häger, K.P., Müller, B., Wind, C., et al., Evolution of legumin genes: loss of an ancestral intron at the beginning of angiosperm diversification, *FEBS Lett.*, 1996, vol. 387, no. 1, pp. 94–98.
https://doi.org/10.1016/0014-5793(96)00477-2
52. Trapp, S.C. and Croteau, R.B., Genomic organization of plant terpene synthases and molecular evolutionary implications, *Genetics*, 2001, vol. 158, no. 2, pp. 811–832.
https://doi.org/10.1093/genetics/158.2.811
53. Jeffares, D.C., Mourier, T., and Penny, D., The biology of intron gain and loss, *Trends Genet.*, 2006, vol. 22, no. 1, pp. 16–22.
https://doi.org/10.1016/j.tig.2005.10.006
54. Ludwig, S.R., Habera, L.F., Dellaporta, S.L., and Wessler, S.R., *Lc*, a member of the maize *R* gene family responsible for tissue-specific anthocyanin production, encodes a protein similar to transcriptional activators and contains the myc-homology region, *Proc. Natl. Acad. Sci. U.S.A.*, 1989, vol. 86, no. 18, pp. 7092–7096.
https://doi.org/10.1073/pnas.86.18.7092
55. Paz-Ares, J., Wienand, U., Peterson, P.A., and Saedler, H., Molecular cloning of the *c* locus of *Zea mays*: a locus regulating the anthocyanin pathway, *EMBO J.*, 1986, vol. 5, no. 5, pp. 829–833.
https://doi.org/10.1002/j.1460-2075.1986.tb04291.x
56. Goff, S.A., Cone, K.C., and Fromm, M.E., Identification of functional domains in the maize transcriptional activator C1: comparison of wild-type and dominant inhibitor proteins, *Genes Dev.*, 1991, vol. 5, no. 2, pp. 298–309.
https://doi.org/10.1101/gad.5.2.298
57. Goff, S.A., Cone, K.C., and Chandler, V.L., Functional analysis of the transcriptional activator encoded by the maize *B* gene: evidence for a direct functional interaction between two classes of regulatory proteins, *Genes Dev.*, 1992, vol. 6, no. 5, pp. 864–875.
https://doi.org/10.1101/gad.6.5.864
58. Roth, B.A., Goff, S.A., Klein, T.M., and Fromm, M.E., C1- and R-dependent expression of the maize *Bz1* gene requires sequences with homology to mammalian myb and myc binding sites, *Plant Cell*, 1991, vol. 3, no. 3, pp. 317–325.
https://doi.org/10.1105/tpc.3.3.317
59. Radicella, J.P., Turks, D., and Chandler, V.L., Cloning and nucleotide sequence of a cDNA encoding B-Peru, a regulatory protein of the anthocyanin pathway in maize, *Plant Mol. Biol.*, 1991, vol. 17, no. 1, pp. 127–130.
https://doi.org/10.1007/BF00036813
60. Selinger, D.A. and Chandler, V.L., A mutation in the *pale aleurone color1* gene identifies a novel regulator of the maize anthocyanin pathway, *Plant Cell*, 1999, vol. 11, no. 1, pp. 5–14.
https://doi.org/10.1105/tpc.11.1.5
61. Zhang, F., Gonzalez, A., Zhao, M., et al., A network of redundant bHLH proteins functions in all TTG1-dependent pathways of *Arabidopsis, Development*, 2003, vol. 130, no. 20, pp. 4859–4869.
https://doi.org/10.1242/dev.00681

62. Gonzalez, A., Zhao, M., Leavitt, J.M., and Lloyd, A.M., Regulation of the anthocyanin biosynthetic pathway by the TTG1/bHLH/Myb transcriptional complex in *Arabidopsis* seedlings, *Plant J.*, 2008, vol. 53, no. 5, pp. 814–827. https://doi.org/10.1111/j.1365-313X.2007.03373.x

63. Baudry, A., Heim, M.A., Dubreucq, B., et al., TT2, TT8, and TTG1 synergistically specify the expression of *BANYULS* and proanthocyanidin biosynthesis in *Arabidopsis thaliana, Plant J.*, 2004, vol. 39, no. 3, pp. 366–380. https://doi.org/10.1111/j.1365-313X.2004.02138.x

64. Payne, C.T., Zhang, F., and Lloyd, A.M., GL3 encodes a bHLH protein that regulates trichome development in *Arabidopsis* through interaction with GL1 and TTG1, *Genetics*, 2000, vol. 156, no. 3, pp. 1349–1362. https://doi.org/10.1093/genetics/156.3.1349

65. Baudry, A., Caboche, M., Lepiniec, L., TT8 controls its own expression in a feedback regulation involving TTG1 and homologous MYB and bHLH factors, allowing a strong and cell-specific accumulation of flavonoids in *Arabidopsis thaliana, Plant J.*, 2006, vol. 46, no. 5, pp. 768–779. https://doi.org/10.1111/j.1365-313X.2006.02733.x

66. Radicella, J.P., Brown, D., Tolar, L.A., and Chandler, V.L., Allelic diversity of the maize *B* regulatory gene: different leader and promoter sequences of two *B* alleles determine distinct tissue specificities of anthocyanin production, *Genes Dev.*, 1992, vol. 6, no. 11, pp. 2152–2164. https://doi.org/10.1101/gad.6.11.2152

67. Albert, N.W., Butelli, E., Moss, S.M.A., et al., Discrete bHLH transcription factors play functionally overlapping roles in pigmentation patterning in flowers of *Antirrhinum majus, New Phytol.*, 2021, vol. 231, no. 2, pp. 849–863. https://doi.org/10.1111/nph.17142

68. Walker, E.L., Robbins, T.P., Bureau, T.E., et al., Transposon-mediated chromosomal rearrangements and gene duplications in the formation of the maize *R-r* complex, *EMBO J.*, 1995, vol. 14, no. 10, pp. 2350–2363. https://doi.org/10.1002/j.1460-2075.1995.tb07230.x

69. Kermicle, J.L., Somatic and meiotic instability of *R-stippled*, an aleurone spotting factor in maize, *Genetics*, 1970, vol. 64, no. 2, pp. 247–258. https://doi.org/10.1093/genetics/64.2.247

70. Robbins, T.P., Walker, E.L., Kermicle, J.L., et al., Meiotic instability of the *R-r* complex arising from displaced intragenic exchange and intrachromosomal rearrangement, *Genetics*, 1991, vol. 129, no. 1, pp. 271–283. https://doi.org/10.1093/genetics/129.1.271

71. Harris, L.J., Currie, K., and Chandler, V.L., Large tandem duplication associated with a *Mu2* insertion in *Zea mays B-Peru* gene, *Plant Mol. Biol.*, 1994, vol. 25, no. 5, pp. 817–828. https://doi.org/10.1007/BF00028876

72. Selinger, D.A. and Chandler, V.L., *B-Bolivia*, an allele of the maize *b1* gene with variable expression, contains a high copy retrotransposon-related sequence immediately upstream, *Plant Physiol.*, 2001, vol. 125, no. 3, pp. 1363–1379. https://doi.org/10.1104/pp.125.3.1363

73. Zimmermann, I.M., Heim, M.A., Weisshaar, B., and Uhrig, J.F., Comprehensive identification of *Arabidopsis thaliana* MYB transcription factors interacting with R/B-like BHLH proteins, *Plant J.*, 2004, vol. 40, no. 1, pp. 22–34. https://doi.org/10.1111/j.1365-313X.2004.02183.x

74. Strygina, K.V., Börner, A., and Khlestkina, E.K., Identification and characterization of regulatory network components for anthocyanin synthesis in barley aleurone, *BMC Plant Biol.*, 2017, vol. 17, suppl. 1, p. 184. https://doi.org/10.1186/s12870-017-1122-3

75. Zeven, A.C., Wheats with purple and blue grains: a review, *Euphytica*, 1991, vol. 56, no. 3, pp. 243–258. https://doi.org/10.1007/BF00042371

76. Li, N., Li, S., Zhang, K., et al., *ThMYC4E*, candidate *Blue aleurone 1* gene controlling the associated trait in *Triticum aestivum, PLoS One*, 2017, vol. 12, no. 7, pp. 1–13. https://doi.org/10.1371/journal.pone.0181116

77. Liu, X., Zhang, M., Jiang, X., et al., *TbMYC4A* is a candidate gene controlling the blue aleurone trait in a wheat–*Triticum boeoticum* substitution line, *Front. Plant Sci.*, 2021, vol. 12, p. 762265. https://doi.org/10.3389/fpls.2021.762265

78. Martis, M.M., Zhou, R., Haseneyer, G., et al., Reticulate evolution of the rye genome, *Plant Cell*, 2013, vol. 25, no. 10, pp. 3685–3698. https://doi.org/10.1105/tpc.113.114553

79. Hanson, M.A., Gaut, B.S., Stec, A.O., et al., Evolution of anthocyanin biosynthesis in maize kernels: the role of regulatory and enzymatic loci, *Genetics*, 1996, vol. 143, no. 3, pp. 1395–1407. https://doi.org/10.1093/genetics/143.3.1395

80. Petroni, K., Pilu, R., and Tonelli, C., Anthocyanins in corn: a wealth of genes for human health, *Planta*, 2014, vol. 240, no. 5, pp. 901–911. https://doi.org/10.1007/S00425-014-2131-1

81. Sweeney, M.T., Thomson, M.J., Pfeil, B.E., and McCouch, S., Caught red-handed: *Rc* encodes a basic helix-loop-helix protein conditioning red pericarp in rice, *Plant Cell*, 2006, vol. 18, no. 2, pp. 283–294. https://doi.org/10.1105/tpc.105.038430

82. Himi, E., Nisar, A., and Noda, K., Colour genes (*R* and *Rc*) for grain and coleoptile upregulate flavonoid biosynthesis genes in wheat, *Genome*, 2005, vol. 48, no. 4, pp. 747–754. https://doi.org/10.1139/G05-026

83. Himi, E., Maekawa, M., Miura, H., and Noda, K., Development of PCR markers for *Tamyb10* related to *R-1*, red grain color gene in wheat, *Theor. Appl. Genet.*, 2011, vol. 122, no. 8, pp. 1561–1576. https://doi.org/10.1007/s00122-011-1555-2

84. Oikawa, T., Maeda, H., Oguchi, T., et al., The birth of a black rice gene and its local spread by introgression, *Plant Cell*, 2015, vol. 27, no. 9, pp. 2401–2414. https://doi.org/10.1105/tpc.15.00310

85. Kim, D.H., Yang, J.H., Ha, S.H., et al., An OsKala3, R2R3 MYB TF, is a common key player for black rice pericarp as main partner of an OsKala4, bHLH TF,

Front. Plant Sci., 2021, vol. 12, p. 765049. https://doi.org/10.3389/fpls.2021.765049

86. Stetter, M.G., Vidal-Villarejo, M., and Schmid, K.J., Parallel seed color adaptation during multiple domestication attempts of an ancient new world grain, *Mol. Biol. Evol.*, 2020, vol. 37, no. 5, pp. 1407–1419. https://doi.org/10.1093/molbev/msz304/5682418
87. Tereshchenko, O.Y., Arbuzova, V.S., and Khlestkina, E.K., Allelic state of the genes conferring purple pigmentation in different wheat organs predetermines transcriptional activity of the anthocyanin biosynthesis structural genes, *J. Cereal Sci.*, 2013, vol. 57, no. 1, pp. 10–13. https://doi.org/10.1016/j.jcs.2012.09.010
88. Tereshchenko, O.Y., Gordeeva, E.I., Arbuzova, V.S., et al., The D genome carries a gene determining purple grain colour in wheat, *Cereal Res. Commun.*, 2012, vol. 40, no. 3, pp. 334–341. https://doi.org/10.1556/crc.40.2012.3.2
89. Shoeva, O.Y., Gordeeva, E.I., and Khlestkina, E.K., The regulation of anthocyanin synthesis in the wheat pericarp, *Molecules*, 2014, vol. 19, no. 12, pp. 20266–20279. https://doi.org/10.3390/molecules191220266
90. Jiang, W., Liu, T., Nan, W., et al., Two transcription factors TaPpm1 and TaPpb1 co-regulate anthocyanin biosynthesis in purple pericarps of wheat, *J. Exp. Bot.*, 2018, vol. 69, no. 10, pp. 2555–2567. https://doi.org/10.1093/jxb/ery101
91. Zong, Y., Xi, X., Li, S., et al., Allelic variation and transcriptional isoforms of wheat *TaMYC1* gene regulating anthocyanin synthesis in pericarp, *Front. Plant Sci.*, 2017, vol. 8, p. 295427. https://doi.org/10.3389/fpls.2017.01645
92. Shoeva, O.Y., Mock, H.P., Kukoeva, T.V., et al., Regulation of the flavonoid biosynthesis pathway genes in purple and black grains of *Hordeum vulgare, PLoS One*, 2016, vol. 11, no. 10, p. e0163782. https://doi.org/10.1371/journal.pone.0163782
93. Cockram, J., White, J., Zuluaga, D.L., et al., Genome-wide association mapping to candidate polymorphism resolution in the unsequenced barley genome, *Proc. Natl. Acad. Sci. U.S.A.*, 2010, vol. 107, no. 50, pp. 21611–21616. https://doi.org/10.1073/pnas.1010179107
94. Gordeeva, E.I., Glagoleva, A.Y., Kukoeva, T.V., et al., Purple-grained barley (*Hordeum vulgare* L.): marker-assisted development of NILs for investigating peculiarities of the anthocyanin biosynthesis regulatory network, *BMC Plant Biol.*, 2019, vol. 19, suppl. 1, p. 52. https://doi.org/10.1186/s12870-019-1638-9
95. Antropov, V.I. and Antropov, V.F., Rye–*Secale* L., in *Kul'turnaya flora SSSR* (Cultivated Flora of the Soviet Union), vol. 2: *Khlebnye zlaki* (Cereals), Leningrad: Gos. Izd. Sovkhoznoi i Kolkhoznoi Lit., 1936, pp. 3–95.
96. Smirnov, V.G. and Sosnikhina, S.P., *Genetika rzhi* (Genetics of Rye), Leningrad: Leningrad. Univ., 1984.
97. de Vries, J.N. and Sybenga, J., Chromosomal location of 17 monogenically inherited morphological markers in rye (*Secale cereale* L.) using the translocation tester set, *Pflanzenzüchtung*, 1984, vol. 192, pp. 177–139.
98. Raviv, B., Godwin, J., Granot, G., and Grafi, G., The dead can nurture: novel insights into the function of dead organs enclosing embryos, *Int. J. Mol. Sci.*, 2018, vol. 19, no. 8, p. 2455. https://doi.org/10.3390/ijms19082455
99. Lascoux, M., Glémin, S., and Savolainen, O., Local adaptation in plants, *eLS*, 2016, pp. 1–7. https://doi.org/10.1002/9780470015902.a0025270
100. Dwivedi, S.L., Mattoo, A.K., Garg, M., et al., Developing germplasm and promoting consumption of anthocyanin-rich grains for health benefits, *Front. Sustainable Food Syst.*, 2022, vol. 6, p. 867897. https://doi.org/10.3389/fsufs.2022.867897
101. Gordeeva, E., Shoeva, O., Mursalimov, S., et al., Fine points of marker-assisted pyramiding of anthocyanin biosynthesis regulatory genes for the creation of black-grained bread wheat (*Triticum aestivum* L.) lines, *Agronomy*, 2022, vol. 12, no. 12, p. 2934. https://doi.org/10.3390/agronomy12122934
102. Casas, M.I., Duarte, S., Doseff, A.I., and Grotewold, E., Flavone-rich maize: an opportunity to improve the nutritional value of an important commodity crop, *Front. Plant Sci.*, 2014, vol. 5, p. 440. https://doi.org/10.3389/fpls.2014.00440
103. Glagoleva, A., Kukoeva, T., Mursalimov, S., et al., Effects of combining the genes controlling anthocyanin and melanin synthesis in the barley grain on pigment accumulation and plant development, *Agronomy*, 2022, vol. 12, no. 1, p. 112. https://doi.org/10.3390/agronomy12010112