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STRESS METABOLITES IN WHEAT: ROLE IN ADAPTATION TO DROUGHT

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Drought is one of the main factors limiting agricultural production and leading to crop losses. Wheat, being a source of food for over half of the world's population, is a plant species that is very susceptible to drought. In this regard, research into the species-specific stress metabolites and physiological and biochemical mechanisms of drought tolerance is of particular practical interest. This review comprehensively examines the roles of soluble carbohydrates, proline, polyamines, and GABA, as well as their functional interplay, in adaptation of wheat and other plant species to drought.

Keywords: drought, Triticum aestivum, stress metabolites, soluble carbohydrates, proline, polyamines, gamma-aminobutyric acid, osmoprotectors, antioxidants, cellular signaling.

Drought is one of the most common abiotic stressors affecting plants. Currently, arid zones occupy 40% of the world's land area; drought regularly affects at least 70 countries worldwide [1]. A continuous upward trend in the frequency and intensity of severe droughts has been observed worldwide since the 1970s, with the global dryland area increasing by at least 1% annually [2]. Current models predict that this trend will continue for at least several decades [3].

Among abiotic stresses, drought is one of the major factors limiting crop production: the drought-caused yield loss exceeds the cumulative loss related to damage of all pathogens to crops [4]. Water deficit during plant growth reduces the vegetative mass accumulation and disrupts the formation of generative organs, while water deficit during seed germination suppresses seedling emergence, and even moderate drought can prevent the genetic yield potential fulfilment [5]. In this regard, increasing the drought tolerance of agricultural crops can become one of the most urgent challenges in experimental plant biology and related scientific fields for many decades. A better understanding of the physiological, biochemical and molecular mechanisms that allow crops to withstand periods of water deficit is the key to the development of both breeding-genetic approaches to boosting drought tolerance and biotechnological tools associated with the application of a wide assortment of exogenous compounds – tolerance inducers [6].

Drought tolerance is a result of the functioning of numerous interrelated mechanisms in plants operating at the molecule, cell and organism levels [7]. These mechanisms are initiated due to the recognition of changes in the water balance by sensors and the transmission of a signal to the genetic apparatus through signal pathways. Along with this, hormonal changes occur, information about which is also transmitted to the genome by signaling mediators and leads to the activation of specific transcription factors, reprogramming the expression of many hundreds of genes and enhancing the functioning of corresponding stress-protective systems [8-10]. The systems of osmotic regulation, antioxidant protection and synthesis of specific proteins, primarily dehydrins and aquaporins, are thought to be the most important ones [11-14]. Moreover, many components of these systems are multifunctional. In particular, osmolytes, including those that can accumulate in plant cells in large amounts (proline, soluble carbohydrates), simultaneously perform antioxidant, chaperone and membrane-protective functions [15]. Well-known enzymatic (superoxide dismutase (SOD), various peroxidases) and non-enzymatic (primarily glutathione) components of the antioxidant system are involved not only in the antioxidant protection of biomacromolecules but also in redox signaling and redox regulation [14]. Dehydrins are highly hydrophilic proteins and, along with osmolytes, enhance the water-holding capacity of cells and participate in the neutralization of reactive oxygen species (ROS) [16-18].

Among the listed mechanisms, the accumulation of osmolytes is considered as one of the most informative markers of the tolerance of plant genotypes [5]. In several studies, it was shown that this process is closely related to maintaining yields by plants under arid conditions and other indicators of field drought tolerance in different species [19-21]. It can be attributed to the multifunctionality of stress metabolites [22], which are (rather conditionally) called osmolytes. Along with multifunctionality, another important feature of these compounds is their complex metabolic and functional interrelationships [23]. This aspect is becoming a new research trend in the rather old topic of osmolyte accumulation by plants during drought. Continuous expansion of the list of compounds that are categorized as stress metabolites and, in part, osmolytes, is another important aspect [24-26]. Progress in studying osmolytes and stress metabolites in general is currently largely associated with the expanded use of metabolomics methods [27, 28]. A comprehensive insight into the role of the accumulation of different stress metabolites and their functional interactions with one another in plant adaptation to drought has become the primary goal of this review.

Despite the fact that various stress metabolites accumulated in different plant taxonomic groups in response to drought stress are very similar, the strategies of biochemical adaptation of different species have certain specificity. In this regard, we limited the scope of this review to discussing the peculiarities of accumulation of polyfunctional osmolytes in one of the most important cereals - bread wheat [21, 29]. Wheat, being one of the most important crops affecting global food security, is known as a food source for more than 50% of the world's population [30]. At the same time, wheat is classed as “very sensitive” to water deficit [31]. In the meanwhile, 50% of the wheat-sown lands are exposed to drought [29]. However, there are many examples demonstrating possibilities of improving the wheat’s capacity for osmoregulation associated with the accumulation of metabolites osmoprotective properties by means of selection, crossing and transgenesis [21]. These facts indicate the need to analyze new information on the functions of stress metabolites and their roles in the drought adaptation strategy of wheat. Nevertheless, despite the prevalence and economic importance of this species, the mechanisms of its drought tolerance remain unclear. This prompted us, in some cases, to turn to data obtained in other plant species when considering the physiological roles and transformations of stress metabolites.

Soluble Carbohydrates

General information on stress-protective functions of sugars in plants. Carbohydrates produced in photosynthesis are major metabolic and energy resources of plants [32]. Drought can not only trigger the accumulation of sugars but also promote the decomposition of storage carbohydrates (primarily starch) into soluble carbohydrates, leading to a decrease in the cellular water potential, which is believed to be an effective strategy to absorb the limited moisture that is available in soil [33]. Numerous studies showed that exposure of plants to abiotic stressors could enhance the formation of non-structural carbohydrates such as sucrose, maltose, trehalose, lactose, monosaccharides, and some polyols (mannitol, sorbitol) [34-36]. These low-molecular compounds significantly contribute to maintaining the osmotic pressure in cells. In Arabidopsis plants, it was demonstrated that soluble carbohydrates under arid conditions made a greater contribution to cellular osmotic adjustments compared to the proline contribution, which is recognized as one of the major osmolytes in plant cells [37]. In addition, soluble carbohydrates are involved in antioxidant protection and maintaining the stability of proteins and membranes [35]. Thus, it has long been known that sucrose can replace water in phospholipid structures exposed to cell dehydration- induced by stressors [38]. It was experimentally proven that hydrogen bonds between oxygen atoms of phosphates in phospholipids and between hydrogen atoms in hydroxyls of sugars, respectively, can be formed [39] and contribute to maintain the structure of membranes exposed to damaging factors.

In recent decades, sugars have been considered not only as metabolites but also as signaling mediators. It is assumed that sugar-transporting proteins can act as sugar sensors. For example, the transporter protein AtSUT2/SUC3, which is localized in the plasma membrane, is discussed as a possible sensor of sucrose [33]. On the other hand, it is hypothesized that the sucrose signal is formed after its conversion by invertase into glucose and fructose. Hexokinases can be sensors of these signals [40]. When the glucose concentration in the cell increases, nuclear hexokinase forms a complex involved in the suppression of photosynthesis. In addition, G proteins, which transmit a signal impulse to hexokinases, are considered as extracellular sensors of soluble carbohydrates. The latter are thought to be transporters of sugars to organelles [33]. The most interesting question is what adaptive responses to drought can be induced by sugar signals. There are very few specific examples of such responses so far. Data on the induction of anthocyanin synthesis in Arabidopsis and wheat by a sucrose-triggered signal under drought and some biotic stresses seem quite convincing [40]. Along with anthocyanins, which perform antioxidant and other stress-protective functions, the sucrose signal can probably induce the synthesis of fructans, which are especially important for adaptation to abiotic stresses (see below). Calcium ions and transcription factors of the MYB family are involved in these signaling pathways [40]. Another signaling pathway associated with soluble carbohydrates suggests the participation of the protein kinase SnRK1. Glucose and its derivatives, such as glucose-6-phosphate and trehalose-6-phosphate, were shown to interact with SnRK1 [33]. SnRK1 is known to regulate the expression of genes encoding thousands of proteins, including transcription factors, vital metabolic enzymes, and chromatin remodeling proteins [41]. SnRK1 is also known as a sensor of ABA, one of the major stress phytohormones, and it can affect other hormones and signaling molecules-gasotransmitters involved in stomatal closure in response to drought [42]. However, possible interactions between these signals and soluble carbohydrate signals remain poorly studied and are beyond the scope of this review.

All soluble carbohydrates are likely to protective functions to one degree or another under osmotic stress. However, there is experimental evidence suggesting specific functions of some sugars. Thus, it was shown that the disaccharides sucrose, trehalose, maltose and lactose can significantly neutralize free radicals *in vitro*, inhibiting the Fenton reaction [43]. Fructans have even higher antioxidant activities [44]. They stabilize membranes, eliminating hydroxyl radicals and thereby preventing lipid peroxidation. At the same time, the participation of monosaccharides as direct antioxidants seems to be unlikely. It is assumed that they contribute to antioxidant protection through their polymers or act as secondary messengers influencing gene expression and syntheses and activities of other antioxidants [45]. Nevertheless, there are publications on the prevention of osmotic stress-induced lipid peroxidation (LPO) via the treatment of wheat plants with exogenous glucose in low concentrations [46, 47]. However, it remains unclear whether glucose acts as a direct scavenger of ROS or its influence on the development of oxidative stress is mediated. In this regard, it should be noted that exogenous glucose may increase the activities of key antioxidant enzymes (SOD, catalase, and peroxidase) in wheat plants [46].

In recent years, information on the potent stress-protective effects of some sugars, in particular, trehalose and raffinose, has been intensively accumulated. It is hypothesized that these compounds can exhibit specific physiological activities different from those of other carbohydrates [35].

Trehalose is a non-reducing disaccharide consisting of two glucose residues linked by an α, α-1,1-glycosidic bond. Its unique chemical properties and, as a result, biological functions are thought to be a result of the participation of both reducing groups in the creation of a glycosidic bond in the trehalose molecule [48]. Unlike other disaccharides, trehalose is resistant to acid hydrolysis and remains stable in acidic solutions when heated [49]. Trehalose is highly hydrophilic due to its inability to form internal hydrogen bonds [48]. In case of dehydration, it creates hydrogen bonds between its OH groups and polar groups of proteins and phosphate groups of membrane lipids. It is believed that trehalose is able to protect biomolecules against denaturation even upon extreme dehydration and to restore their functional activities upon rehydration [48]. It was also shown that trehalose could serve as an acceptor that captures and neutralizes free radicals [50]. Thus, it, together with other carbohydrates, can be a component of the antioxidant system that eliminates radical ROS. As exemplified in wheat plants, treatment with exogenous trehalose mitigated the adverse effects of high-temperature stress due to the binding of the superoxide anion radical and hydrogen peroxide [51]. However, it is unclear to which of the mechanisms the trehalose effect on oxidative stress is related more: to its direct interaction with ROS or to stabilization of antioxidant enzymes [48].

Trehalose biosynthesis in plants occurs via two enzyme-catalyzed reactions (Fig. 1). First, uridine diphosphate glucose (UDP-glucose) and glucose-6-phosphate are converted by trehalose-6-phosphate synthase (TPS) to form the intermediate product – trehalose-6-phosphate (T6P). T6P is then converted to trehalose via dephosphorylation by trehalose-6-phosphate phosphatase (TPP) [52]. In recent decades, the genes for these trehalose biosynthesis enzymes (TPS and TPP) from plants and prokaryotes have been successfully used to create transgenic plants of various species, which were proven to be tolerant to drought and other stressors. For example, overexpression of the yeast *ScTPS1* gene in potato [53] and tomato [54] plants led to the accumulation of trehalose and the development of tolerance to salinity, drought, and cold [48, 55]. Transgenic rice plants carrying the *Escherichia coli* TPS gene accumulated 3–10 times more trehalose, which improved growth and reduced photooxidative damage upon exposure to drought, salinity, and low temperatures [56].

Raffinose is a non-reducing trisaccharide consisting of D-galactose, D-glucose, and D-fructose residues. Raffinose is one of the α-galactosyl derivatives of sucrose [57]. The raffinose family of oligosaccharides also includes stachyose and verbascose; they are all formed from sucrose and activated galactose fragments [57].

Raffinose is believed to be an important multifunctional stress metabolite and one of the transport forms of carbohydrates [50]. There is evidence that raffinose and other oligosaccharides of this family play key roles in the tolerance of plants, in particular, the tolerance of cereal seeds to drying [50]. The stress-protective functions of raffinose are associated with several mechanisms: (1) Direct osmoprotective action and ability to maintain the stability of cell membranes under severe dehydration and to prevent membrane fusion after rehydration [58]; (2) Ability to be transported into chloroplasts to protect thylakoids, maintaining photosynthesis under unfavorable conditions [59]; (3) Antioxidant (antiradical) effects [40]. Raffinose molecules were demonstrated to be able to neutralize hydroxyl radicals *in vitro*, forming oxidized monosaccharide products [45].

The biosynthesis of oligosaccharides included in the raffinose family is closely related to the galactinol pathway [60]. Molecular genetic evidence was obtained for the roles of the galactinol synthase and raffinose synthase genes (*GolS* and *RFS)* in raffinose synthesis under osmotic stress, as exemplified in potato [50]. The expression of these genes was significantly enhanced by tissue dehydration. Enhanced expression of the peanut *AdGolS3* gene in Arabidopsis led to an increase in raffinose levels and mitigation of stress symptoms under drought and salinity [61]. In wheat plants, raffinose was shown to be involved in adaptation to repeated droughts [62].

Fructans are another group of soluble carbohydrates that are important for plant adaptation to abiotic stresses, including drought. They are fructose polymers and are synthesized from sucrose molecules. In this case, the fructosyl group is transferred in two stages, which are catalyzed by sucrose:sucrose-1-fructosyltransferase and sucrose:fructan-6-fructosyltransferase [35]. It was shown that under stressful conditions, the expression of genes encoding enzymes for fructose and fructan syntheses was enhanced in leaves [63]. Sugar beet plants carrying genes for bacterial enzymes of fructan biosynthesis demonstrated increased drought tolerance [64]. Fructans are highly soluble in water, thereby stabilizing solutions against crystallization upon cooling [47]. In addition, fructans, like other soluble carbohydrates, stabilize membranes and participate in osmotic regulation. Plants accumulate fructans mainly in vacuoles [35]. However, the exocytosis system originating from tonoplast vesi-

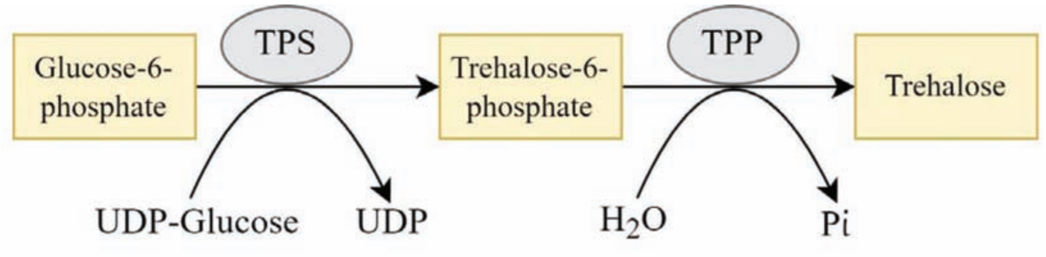

Fig. 1. Synthesis of trehalose in plants. TPS – trehalose-6-phosphate synthase; TPP – trehalose-6-phosphate phosphatase

cles can participate in the transport of fructans from vacuoles to the apoplast, where they act as hydroxyl radical scavengers and simultaneously have a stabilizing effect on membranes, which is very important during drought [45].

There is evidence that fructans can maintain grain filling in drought-exposed wheat. Remobilization of previously stored carbohydrates from stems to caryopses during grain filling is one of the mechanisms of drought tolerance. It was shown that the drought-tolerant wheat genotype was characterized by a significantly higher efficiency of stem reserve remobilization compared to the non-tolerant one [65]. In addition, high fructan levels in peduncles were intrinsic to the drought-tolerant accession under arid conditions, determining more effective protection against oxidative stress and faster fructan remobilization to maintain grain filling. It was shown that under arid conditions during grain filling, the remobilization of assimilates into caryopses was a more effective adaptation strategy than maintaining photosynthesis [66]. In this regard, markers of fructan remobilization from stems to caryopses, including expression levels of genes for fructan synthesis and transport, are proposed to be used to select drought-tolerant genotypes with enhanced remobilization in wheat breeding programs.

Soluble carbohydrate accumulation and adaptation to drought in wheat. Many studies demonstrated an association between soluble carbohydrate content and drought tolerance in wheat. A study of the response of 20 bread wheat cultivars to PEG 6000-simulated drought at early stages of plant development showed an increase in leaf sugar content in both tolerant and susceptible cultivars [67]. However, it should be noted that in this study, sugar content was calculated per fresh weight; thus, inter-cultivar differences attributed to changes in tissue water content were not taken into account. Marcińska et al. [68] investigated the responses of two wheat cultivars to various levels of PEG 6000-simulated drought. The authors observed a significant increase in the sugar content (calculated per dry matter) only in the susceptible cultivar and only under severe simulated drought, which significantly decreased the relative water contents in roots and leaves, causing the development of oxidative stress as well. On the other hand, when comparing the responses of two wheat cultivars differing in drought tolerance to mannitol-induced stress, the researchers revealed that water deficit decreased the starch content but increased the sucrose content in seedlings of the tolerant cultivar compared to the susceptible one [69]. They also showed that drought stress decreased the activity of α-amylase in the endosperm of the seed of both cultivars but increased the activity of α- and β-amylases in shoots of the tolerant cultivar. In addition, higher activities of sucrose phosphate synthetase and invertase were recorded in seedling organs of the tolerant cultivar compared to these parameters in the susceptible one. When comparing the responses of eight wheat cultivars to PEG 8000-induced drought in water culture, the researchers observed an increase in the sugar content in all cultivars [70]. However, in the non-tolerant cultivars, where the relative water content was concomitantly considerably reduced, this effect was much weaker than in the tolerant cultivars.

In most studies comparing the response of wheat genotypes with different drought tolerance, the total content of soluble carbohydrates was assessed. The contributions of their specific chemical forms to drought tolerance have only been investigated in a few studies until now. Thus, when comparing the responses of two wheat cultivars to drought using metabolomics methods, the researchers found that the more tolerant cultivar accumulated trehalose and mannose in significant amounts, while the contents of these carbohydrates in the susceptible cultivar decreased significantly during drought [71].

Leaves of green wheat plants subjected to osmotic stress induced by non-penetrating PEGs or (less often) mannitol are the most common experimental objects in studies of changes in osmolyte contents in response to drought. At the same time, the response of etiolated seedlings to drought remains poorly understood. However, as already noted, this is relevant since wheat can be exposed to drought as early as at seed germination [29]. Our experiments using 4-day-old etiolated seedlings of seven wheat cultivars showed that the most noticeable increase in the sugar content in shoots subjected to PEG 6000-simulated drought occurred in the highly tolerant cultivars of the steppe eco-geographical group [72]. At the same time, the sugar content in the non-tolerant cultivars of the forest-steppe and woodland groups remained almost unchanged. There was a strong positive correlation between the increase in the sugars content and the ability to maintain biomass accumulation under drought ($r = 0.85$). A similar pattern was observed when we compared the responses of green plants of the same cultivars to drought when grown in soil in the laboratory [73]. There was a clear inverse correlation between growth suppression and content of sugars increase values (r = -0.79 and -0.91 for linear growth and biomass accumulation, respectively). There was also a strong inverse correlation between sugar content and an oxidative stress marker, malondialdehyde (MDA) (r = -0.81). Thus, in general, most studies found a positive relationship between the ability of cultivars to accumulate sugars in response to drought and their drought tolerance. Such effects are typical for both green plants and etiolated seedlings. However, more extensive comparative studies of relationships between carbohydrate metabolism and drought tolerance of wheat genotypes at different developmental stages are needed for unambiguous conclusions.

Proline

Metabolism and stress-protective functions of proline in plants. L-proline is an unusual proteinogenic amino acid with a five-membered ring containing a protonated secondary amino group. Due to its rare cyclic structure, proline influences the secondary structure of proteins, making them more rigid [74].

Proline content in plant cells is regulated by biosynthesis-degradation balance, forming a cycle with glutamate as the major source for proline biosynthesis and the end product of its catabolism [75]. In plants, the glutamate pathway of proline biosynthesis is dominant and consists of two successive stages (Fig. 2). In the first step, the bifunctional enzyme Δ^1-pyrroline carboxylate synthetase (P5CS) reduces glutamate to glutamate semialdehyde (GSA), which is spontaneously converted to Δ1-pyrroline carboxylate (P5C), a common intermediate in the metabolisms of proline and ornithine. P5C is then reduced to proline by P5C reductase (P5CR). In most plants, P5CS is encoded by two genes, *P5CS1* and *P5CS2*. There is evidence that the *P5CS1* gene expression is mostly enhanced under water stress [76]. Plant P5CR is encoded by one gene; this enzyme converts P5C to proline using mainly NADPH as an electron donor [74]. These processes occur in the cytoplasm and/or in chloroplasts [77]. The ornithine pathway, where ornithine δ-aminotransferase (OAT) catalyzes the formation of P5C from ornithine, which in turn can be converted to proline, is an alternative pathway for proline synthesis (Fig. 2). In Arabidopsis, the OAT pathway is important for arginine formation but not for proline synthesis [78]. However, it was suggested that OAT might be important for proline accumulation in other plant species exposed to certain abiotic stressors as well as to nitrogen deficit [77].

Proline is catabolized in mitochondria, and its catabolism consists of two oxidative steps sequen-

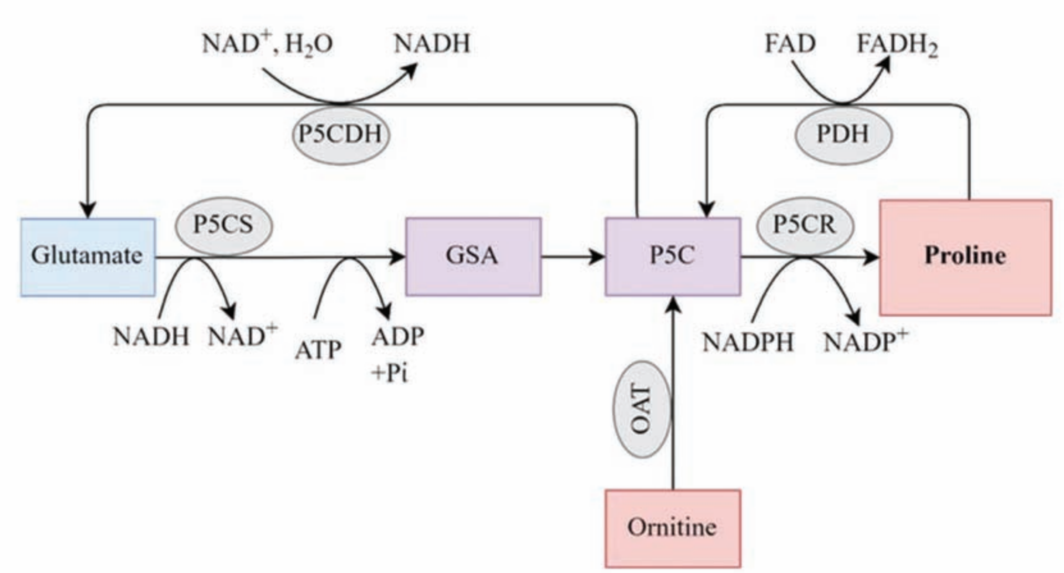

Fig. 2. Synthesis and catabolism of proline in plants. P5CS – Δ^1-pyrroline carboxylate synthase; GSA – glutamate semialdehyde; P5C – Δ^1-pyrroline carboxylate; P5CR – Δ^1-pyrroline carboxylate reductase; OAT – ornithine δ-aminotransferase; PDH – proline dehydrogenase; P5CDH – Δ^1-pyroline carboxylate dehydrogenase. Other explanations in the text

tially catalyzed by proline dehydrogenase (ProDH) and P5C dehydrogenase (P5CDH) [74].

Intracellular L-proline content in plants depends on the intensity of its biosynthesis, catabolism and transport between cells and different intracellular compartments. At the same time, stress-induced accumulation of proline in plant cells exceeds manifold changes in the contents of other stress metabolites. Thus, under normal conditions, its content is below 5% of the total amino acid pool, while under stressful conditions, it can increase to 80% of the total amino acid content in some plant species [79]. In this regard, proline is considered the most common endogenous osmolyte [80]. However, the idea that proline makes a particularly significant contribution to the rise in osmolarity of cell sap has recently been revised. As evidenced by cumulative calculations of data on the total content of all major osmolytes, the contribution of proline to the cell sap osmolarity growth, as a rule, cannot be more than 3-15%, provided that the proline content increases 100-fold (!), which is extremely rare [81]. In this regard, the emphasis is shifted from the osmoregulatory functions of proline to others: antioxidant, chaperone, membrane-protective.

In recent decades, the greatest attention has been paid to the antioxidant effects of proline. Its structural peculiarities give reason to consider the possibility of direct inactivation of radical oxygen species. It was assumed that free proline, as well as its terminal groups in polypeptides, could directly react with hydrogen peroxide and singlet oxygen to form stable free radicals - adducts of proline and hydroxyproline derivatives [82]. However, later, it was shown that proline was not able to inactivate $^1O_2$ in an aqueous medium, which has led to a revision of the assumption about the possible role of proline in protecting plant cells against singlet oxygen under stressful conditions [83]. On the other hand, a direct reaction between hydrogen peroxide and proline is possible at fairly high concentrations of the latter [84], which also calls into question the importance of proline for H_2O_2 detoxification in plant cells.

However, proline may be of great importance in inactivating hydroxyl radical. Signorelli et al. [85, 86] proposed two models to explain the mechanism of this process. According to one of them, the proline molecule alternately binds two hydroxyl radicals, turning into Δ^1-pyrroline-5-carboxylic acid, which is reduced to proline by NADPH-using Δ^1-pyrroline-5-carboxylate reductase. According to another model, hydroxyl attack at the N atom triggers proline decarboxylation. The resulting Δ^1-pyrroline radical then binds the hydroxyl radical, turning into Δ^1-pyrroline. As enzymatic antioxidants are not able to neutralize the hydroxyl radical and proline contents in different compartments are rather high, such a mechanism may be of great importance for protection against free radical damage.

The ability of proline to bind metal ions with variable valence and thereby limit non-enzymatic free radical processes is considered as a separate reason for its antioxidant effect [82]. In addition, chelation of heavy metals by proline reduces their toxic effects as potential inhibitors of antioxidant enzymes, which are also important for preventing the development of oxidative stress [87].

However, proline (both exogenous and endogenous) can have not only an antioxidant, but also a prooxidant effect on plants. Moreover, apparently, the proline-ROS functional interaction can act as a component of cellular redox signaling. In particular, intense oxidation of proline to Δ^1-pyrroline-5-carboxylate by proline dehydrogenase in mitochondria can enhance ROS formation associated with excessive reduction of electron transport chain components [82, 87]. It was suggested that Δ^1-pyrroline-5-carboxylate dehydrogenase (P5CDH) was an important regulator of ROS formation in plants, controlling Δ^1-pyrroline-5-carboxylate amount [87], which is important to avoid overproduction of mitochondrial ROS. In particular, there is evidence that reduced expression of an Arabidopsis *P5CDH* gene homolog, *FIS1*, in flax increased sensitivity to exogenous proline and formation of large amounts of hydrogen peroxide [82].

On the other hand, an increase in proline content may be a signal for activating the expression of antioxidant enzyme genes. Thus, in transformed hybrid Swingle citrumelo (*Citrus paradisi* Macfad. Cv. Duncan × *Poncirus trifoliata* (L.) Raf.) plants, which carried the *P5CS* gene from *Vigna aconitifolia* (VaP5CSF129A) and were noticeable for enhanced proline synthesis, a rise in the expression of genes for different forms of ascorbate peroxidase, Mn-SOD, and chloroplast glutathione reductase was observed under physiologically normal conditions and especially under osmotic stress [88]. The authors believe that in this case proline acts as a signaling/regulatory molecule that changes the expression of genes for antioxidant enzymes. Interestingly, in another study, wheat transformants were also characterized by increased proline contents, but due to the introduction of a double-stranded RNA suppressor of the Arabidopsis proline dehydrogenase gene, there was an increase in the activities of antioxidant enzymes (SOD and ascorbate peroxidase) in chloroplasts [89, 90]. Thus, the effects of proline on gene expression and activities of antioxidant enzymes were demonstrated in different models. Nevertheless, the mechanisms of these effects remain scarcely explored. One can assume that other mediators are involved in these processes, but their nature is still unknown.

Interesting phenomena were also discovered when the effect of exogenous proline on wheat tolerance to PEG 6000-induced osmotic stress was investigated [91]. It was shown that treatment of seedlings with 6 mM proline mitigated the simulated drought-caused growth inhibition and membrane lipid peroxidation and stabilized the chlorophyll content. Exogenous proline also eliminated the phenomenon of decreasing the relative water content in leaves, indicating its osmoregulatory effects. In addition, in the experiments with exogenous proline under simulated drought, the activities of catalase and guaiacol peroxidase increased, but the activity of ascorbate peroxidase did not change [91]. These effects are generally similar to the findings obtained in a study of wheat plants accumulating increased amounts of proline due to suppression of proline dehydrogenase [89]. However, in this case, it is also unclear how proline affects the activities of antioxidant enzymes.

In general, possible signaling functions of proline in stressed plant cells are still discussed as hypothetical models [74, 82, 87]. The involvement of proline in pro- and antioxidant processes is apparently very complex, since its action is multifunctional and can cause both direct and indirect neutralization of ROS, and, on the other hand, create prerequisites for enhanced ROS generation. It is noteworthy that in etiolated seedlings of different wheat cultivars subjected to osmotic stress, a strong direct correlation (r = 0.91) was recorded between proline content and lipid peroxidation product (MDA) amount [92]. Of course, this fact alone does not give grounds to conclude that it was high proline content that caused an intensification of lipid peroxidation. However, when the responses of wheat seedlings of four cultivars differing in drought tolerance to direct inducers of oxidative stress (hydrogen peroxide and iron (II) sulfate) were investigated, it was found that two drought-tolerant cultivars were highly resistant to both oxidative stress inducers, which were recorded as abated growth inhibition and oxidative damage (decreased MDA content) [93]. At the same time, two susceptible cultivars, in which the MDA content was significantly increased and growth was considerably suppressed because of H_2O_2 and $FeSO_4$ treatments, accumulated much more proline compared to the tolerant cultivars under the same conditions. Thus, a close relationship between oxidative stress intensity and proline accumulation can be stated. Moreover, recently, the role of ROS produced by NADPH oxidase in stress-induced proline formation in plant tissues has been shown in different plants by different methods [74, 94, 95].

On the other hand, proline metabolism can affect the intracellular redox status through the proline-pyrroline-5-carboxylate cycle, which can maintain optimal $NADP^+$/NADPH ratios in the cytosol [82]. Proline synthesis is accompanied by $NADP^+$ generation, while proline oxidation generates NADPH. Notably, sufficient $NADP^+$ levels are important to ensure electron transfer to the electron transport chain and prevent its overreduction. This is necessary to minimize ROS generation during stress. At the same time, NADPH produced during proline oxidation can be used to maintain reduced glutathione (GSH) and thioredoxin pools. Thus, the pro- and antioxidant, redox-regulatory and signaling functions of proline are apparently closely intertwined and the causes of switching its roles under different conditions remain to be further investigated.

In addition to its antioxidant protective effect and influence on redox homeostasis and redox signaling, proline can have a direct stabilizing effect on some proteins, acting as a chemical chaperone [87, 96]. Upon significant cell dehydration, proline can act as a water substitute to stabilize cellular structures through hydrophilic interactions and hydrogen bonds. Thus, it forms a hydration shell around unstable proteins and prevents their destruction under stressful conditions [87]. Examples of the stabilizing impact of proline on both plant and animal proteins have been known for a long time. It was shown that proline protected the rabbit muscle M4-lactate dehydrogenase isozyme from denaturation caused by freezing-thawing, high temperatures and chemical agents. The authors suggest that, unlike other osmolytes, proline stabilizes the enzyme not only by enhancing protein hydration but also by interacting with its accessible hydrophobic regions [97]. A protective effect of proline on nitrate reductase in rice plants exposed to osmotic stress was also described [98]. It was reported that proline restored the rice seedling RNase activity *in vitro* after arsenite treatment [99].

Proline and drought tolerance of wheat. Several studies with pairwise comparison of proline content in leaves of drought-tolerant and drought-susceptible wheat cultivars at the vegetative stages of development showed that the proline amount was higher in the tolerant cultivars under stressful conditions [68, 71, 100-103]. At the same time, under normal wetting, no relationships were found between drought tolerance and proline content. Experiments on six wheat cultivars showed that the contents of proline and glycine betaine were much higher in two drought-tolerant cultivars than in the susceptible ones [104]. In addition, there was a direct correlation between the contents of proline and some other free amino acids and sugars in leaves exposed to 10% PEG 8000 and drought tolerance of plants in the early stages of development in a study on 8 simultaneously evaluated cultivars [70]. Similar findings were obtained in an experiment that included a comparison of three drought-tolerant and two susceptible wheat cultivars. The authors reported a more conspicuous increase in the contents of proline and other osmolytes in both leaves and roots of more tolerant genotypes [105]. In an experiment with long-term cultivation of plants on a PEG 6000 solution, it was shown that the proline content in leaves under stress conditions was positively correlated with the aerial part length [67]. A moderate ($r = 0.54$) but significant (at $P \leq 0.05$) correlation was observed due to the large number of correlation pairs in the study ($n = 20$).

However, several other studies did not find a positive relationship between proline accumulation and drought tolerance in bread wheat cultivars. For example, when comparing the response of etiolated seedlings of seven wheat cultivars from different climatic zones to osmotic stress caused by 12% PEG 6000, the researchers reported that proline content was positively correlated with inhibition of seedling growth, although the correlation coefficient was 0.49 and was not significant at $P \leq 0.05$ [72]. At the same time, when imitating drought for green plants grown in soil in the laboratory, the researchers found no correlation between proline accumulation and biomass in the same genotypes (age 6-10 days) [73]. In experiments with a 5-day impact of PEG 6000 on plants pre-grown under physiologically normal conditions, proline content was inversely correlated with aerial part biomass [106]. However, a fairly high correlation coefficient ($r = - 0.55$) was not significant at $P \leq 0.05$ because there were too few correlation pairs.

As already noted, drought tolerance during the generative period is also critical for wheat. The proline contribution to drought tolerance during anthesis and grain filling was studied in few studies until now. It was shown that a 7-day post-anthesis drought caused a greater accumulation of proline in the roots and leaves of the tolerant plants compared to the susceptible ones [107]. When comparing the responses to drought in the generative phase of development of tolerant and susceptible cultivars, the researchers also observed qualitative differences in the composition of amino acids accumulated by wheat plants during drought. It turned out that the tolerant cultivar accumulated more proline, while the susceptible one accumulated asparagine and arginine [108]. Pre-anthesis priming of wheat plants by reduced watering enhanced tolerance to subsequent post-anthesis more severe drought, which was associated with increased expression of the *P5CS* gene and increased activity of this enzyme in leaves [109]. On the other hand, a study on 150 wheat lines exposed to drought during anthesis found a negative correlation between leaf proline content and yield [110].

Thus, when studying proline accumulation by wheat plants at the vegetative and generative stages of development, both positive and negative relationships were recorded between proline accumulation and tolerance, which was assessed by ability to form caryopses. The lack of a direct relationship between proline content and drought tolerance of wheat genotypes may be attributed to the multifunctional role of proline in plants under stressful and normal conditions. One cannot rule out that some of its functions, which are less studied at present (antioxidant, chaperone, signal-regulatory, and others), are not directly related to its total content in tissues. Such functions can be performed due to fine compartmentalization of certain subcellular pools of proline [83] as well as due to dynamic local changes in its content in cells, which is typical for well-studied signaling mediators, in particular calcium and ROS [111, 112].

Polyamines

Metabolism and stress-protective functions of polyamines. Polyamines are aliphatic amino compounds containing unsaturated hydrocarbon chains with two or more primary amino groups and existing as polycations [113]. They are found in a wide variety of eukaryotic and prokaryotic cells [114, 115]. In higher plants, free polyamines are mainly detected, with putrescine, spermidine, and spermine being the most common of them [116]. Polyamines are found in plants and exhibit physiological activities at concentrations of 10^{-9}–10^{-5} M, which exceed concentrations of most phytohormones [117]. Large pools of polyamines are localized in cell walls, vacuoles, chloroplasts, mitochondria, and the nucleus [118].

The polyamine synthesis pathways in plants are well described. Putrescine is the central product of the major pathway of polyamine synthesis; it serves as a metabolite for the syntheses of other polyamines. Putrescine is usually produced from arginine. First, arginine is converted to agmatine by arginine decarboxylase, then agmatine iminohydrolase catalyzes the conversion of agmatine to N-carbamoylputrescine (Fig. 3). Then N-carbamoylputrescine is hydrolyzed by N-carbamoylputrescine amidohydrolase with the release of putrescine, NH_3 and CO_2 [116]. Putrescine biosynthesis can alternatively be accomplished in a single step, i.e., conversion of mitochondrial ornithine to putrescine by ornithine decarboxylase [35] (Fig. 3).

Spermidine and spermine are formed via the sequential addition of aminopropyl first to putrescine and then to spermidine (Fig. 3). These reactions are catalyzed by the corresponding aminopropyltransferases – spermidine synthase and spermine synthase [114, 119].

Polyamines are catabolized by oxidative deamination catalyzed by diamine oxide and polyamine oxidase [35, 116]. When putrescine is converted by diamine oxidase, 4-aminobutanal is formed, and ammonia and hydrogen peroxide are released. 4-Aminobutanal then cyclizes to form pyrroline, which is converted to γ-aminobutyric acid (GABA). Spermidine and spermine are oxidized by polyamine oxidase to form 4-aminobutanal, 3-aminopropyl-4-aminobutanal, 1,3-diaminopropane, NH_3, and H_2O_2 [116].

Polyamines are involved in the regulation of a wide range of plant functions, in particular, cell division and differentiation, rhizogenesis, anthesis, growth, development, and fruit ripening [113, 120]. The stabilizing effects of polyamines on proteins, nucleic acids, and membrane structures, primarily due to their cationic state under physiological conditions are considered as a separate function [118, 121]. They can bind to phospholipid “heads” of membranes, influencing their permeability. Polyamines can also bind to different proteins and affect their functional activities. The binding of polyamines to chromatin can change the accessibility of genomic sites for DNA and RNA polymerases, altering DNA and RNA syntheses [113].

Polyamines play a special role in plant tolerance to abiotic stresses. An increase in polyamine con-

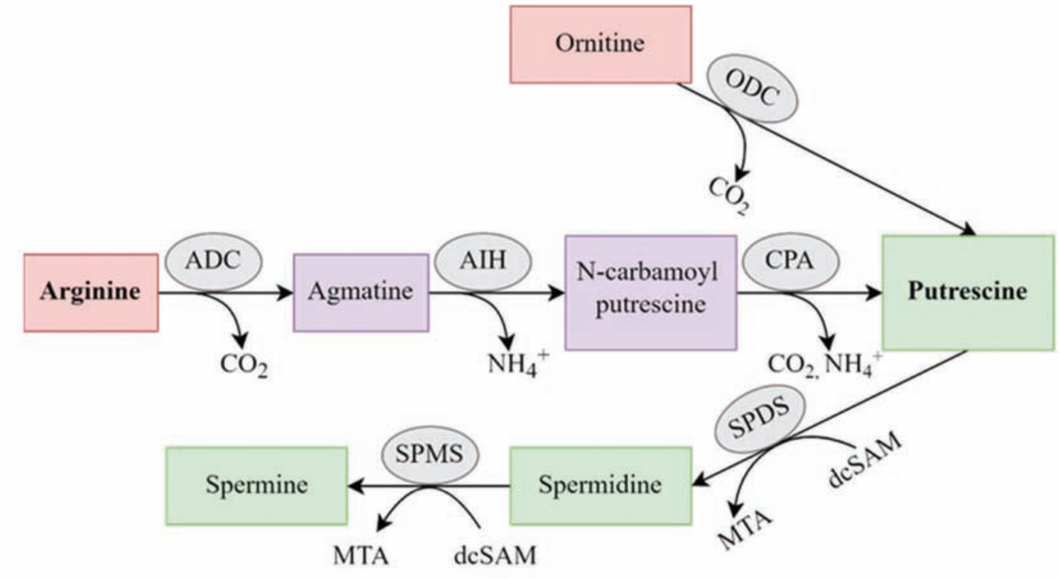

Fig. 3. Synthesis of polyamines in plants. ADC – arginine decarboxylase; AIH – agmatine iminohydrolase; CPA – N-carbamoyl putrescine amidohydrolase; dcSAM – decarboxylated S-adenosylmethionine; MTA – 5-methylthioadenosine; SPDS, spermidine synthase; SPMS, spermine synthase. Other explanations in the text

tent is one of the most pronounced changes in the plant metabolome in response to drought, salt stress, extreme temperatures, hypoxia, ozone, UV, heavy metals, and herbicides [122].

At present, some mechanisms of the stress-protective effects of polyamines in plant cells have been elucidated. In addition to the above-mentioned stabilizing effects on biomacromolecules and membrane structures, polyamines can exert direct and indirect antioxidant effects [123], induce the expression of stress protein genes [124], and participate in the regulation of osmolyte synthesis [113]. All these effects are important for plant adaptation to drought. In addition, experiments with exogenous polyamines showed their ability to induce stomatal closure [125].

New ideas have been recently formed about the roles of polyamines in plant adaptation, specifically the roles associated with polyamines' involvement in cellular signaling pathways [114, 117]. They are attributed to the production of such important signaling molecules as hydrogen peroxide and, probably, nitric oxide during oxidative degradation of polyamines, as well as to the ability of polyamines to directly affect the state of ion (including calcium) channels [120].

Antioxidant function is thought to be an important stress-protective function of polyamines. Many researchers class polyamines as water-soluble compounds with antioxidant properties, along with glutathione, ascorbate, polyphenols and proline [126-128]. Polyamine binding of radical ROS is assumed to be a component of the direct protective effect of polyamines under stressful conditions [129]. Spermidine and spermine are recognized as the most active scavengers of oxygen radicals [128]. The antioxidant properties of polyamines may be attributed to the ease of oxygen-dependent auto- and enzymatic (by di- and polyamine oxidases) oxidation of amino groups.

Inhibition of NADPH oxidase-dependent ROS generation by polyamines may be another mechanism of polyamine abatement of oxidative stress [130]. Putrescine and spermidine were reported to be capable of such inhibition [130-132]. However, opposite effects were also observed. In Arabidopsis, exogenous polyamines were shown to induce an NADPH oxidase-dependent stimulation of oxygen consumption, with spermidine having the strongest effect [133]. This increase is attenuated by treatment with an NADPH oxidase inhibitor, diphenyleneiodonium iodide. It is possible that the modulation of NADPH oxidase activity by polyamines changes over time. Thus, it was demonstrated that the treatment of wheat seedlings with putrescine caused a transient increase in the hydrogen peroxide content in the roots of wheat seedlings [134]. This effect was eliminated by both an NADPH oxidase inhibitor, imidazole, and a diamine oxidase inhibitor, aminoguanidine. Moreover, the treatment of wheat seedlings with putrescine caused an increase in the activities of SOD and catalase and this phenomenon was eliminated by NADPH oxidase and diamine oxidase inhibitors as well as by a hydrogen peroxide scavenger, dimethylthiourea [134]. Probably, exogenous putrescine, causing hydrogen peroxide production by activating NADPH oxidase and diamine oxidase, generates a signal that activates components of the antioxidant system. The role of polyamines in regulating the expression of genes encoding antioxidant enzymes is also evidenced by findings on the antioxidant system of transformants with increased levels of polyamines. Thus, in tobacco plants transformed with a gene for one of the polyamine synthesis enzymes (S-adenosylmethionine decarboxylase), an enhancement in the expression of genes for Mn-SOD, ascorbate peroxidase, catalase, and glutathione-S-transferase genes was observed [135, 136]. These facts indicate the involvement of polyamines in regulating the expression of genes encoding antioxidant system enzymes. However, the mechanisms of such effects remain unexplored. In particular, the question of which signaling mediators, in addition to ROS, can participate in the impact of polyamines on the expression of genes for antioxidant enzymes remains open.

Protection of proteins, including antioxidant enzymes, against denaturing damage and membrane-protective effects may be a separate mechanism of the antioxidant action of polyamines [117]. However, when discussing the antioxidant action of polyamines, one cannot fail to mention their obvious prooxidant effects. As already noted, hydrogen peroxide is generated during oxidative degradation of polyamines. In this regard, the issues of how great the contribution of H_2O_2 generated during polyamine catabolism is to damage inflicted by abiotic stressors and how this relates to the signaling functions of hydrogen peroxide are discussed [137].

In general, one can state that polyamines are involved in different aspects of redox regulation in plant cells: they can directly bind ROS, be their sources, affect the expression of genes for antioxidant enzymes, and exert protective and regulatory effects on specific proteins. To understand their mechanisms of action, research into the specificity of the effects of individual polyamines and clarification of their relationships with other signaling mediators remains relevant.

Polyamines and wheat adaptation to drought. There are still few comparative studies of changes in polyamine levels in different wheat genotypes in response to drought. Doneva et al. [138] showed that the constitutive contents of putrescine and spermidine were higher in the leaves of 7-day-old plants of a tolerant wheat cultivar than those in the leaves of a susceptible one. No differences in the basal spermine content were detected between the cultivars. Under PEG-induced drought, the contents of putrescine and spermidine decreased in both cultivars, while the spermine content increased. The authors believe that a proline pool was used in spermine synthesis.

When the content of polyamines in the leaves of five wheat cultivars was determined at the third leaf stage, it turned out that the basic contents of putrescine, spermidine and spermine were very similar [139]. In response to PEG-induced drought in water culture, there was a 5- to 6-fold rise in the putrescine amount, a 1.5- to 2-fold rise in the spermidine level and a 20- to 40-fold increase in the spermine amount. Moreover, when exposed to drought, more drought-tolerant cultivars increased the contents of all polyamines more conspicuously than non-tolerant ones. Similar but less pronounced changes in the contents of polyamines were noted in roots [139].

Somewhat different results were obtained in an experiment comparing changes in the contents of polyamines in leaves in response to drought at later stages of development [140]. The authors found a significant increase in the putrescine content regardless of how tolerant wheat cultivars were. At the same time, the contents of spermidine and spermine increased more significantly in drought-tolerant cultivars. Moreover, there was a decline in the amounts of these polyamines in the least tolerant cultivar after prolonged drought stress [140].

Determination of the contents of polyamines in leaves of wheat cultivars differing in drought tolerance during the post-pollination phases and grain filling demonstrated an increase in the putrescine content in all cultivars, but it was more noticeable in the susceptible and moderately tolerant cultivars compared to highly tolerant ones. All cultivars also had increased levels of spermidine and spermine in leaves [141]. In another study, where the effect of drought was investigated during the generative phase of wheat development, it was found that the amounts of bound forms of putrescine and spermidine in developing seed embryos increased more noticeably in the more drought-tolerant cultivar than in the susceptible one [142].

Liu et al. [143] showed that drought caused a more significant increase in the spermidine and spermine contents in kernels of the tolerant wheat cultivar at the grain ripening stage, while the putrescine content decreased in both cultivars.

Increased levels of polyamines in response to drought during the reproductive period were also recorded in some other cereals, in particular, in rice [144]. In a comparative study of the responses of six cultivars differing in tolerance to drought, it was found that an increase in contents of free spermidine and spermine as well as of bound putrescine in leaves under water stress was significantly correlated with yield maintenance coefficient (ratio of grain yield under stress to grain yield under normal wetting). At the same time, free putrescine content was positively correlated with yield maintenance coefficient only at the early stage of water stress, while at a later stage, the correlation became negative. These findings suggest that interconversions of polyamines at different stages of the stress response are important for the drought tolerance of plants.

In general, the available data do not yet allow us to give an unambiguous assessment of the relationship between the contents of individual polyamines and the drought tolerance of cereals. Difficulties in elucidating such relationships are explained by intensive interconversions of polyamines, transient nature of changes in their contents and, probably, specific changes in the compartmentation of polyamines in cells, tissues and organs in response to stress. For example, when the compartmentation of polyamines was investigated in wheat subjected to drought, it was shown that the putrescine content increased to a greater extent in leaves. At the same time, spermine was not detected in roots at all [145].

The use of exogenous polyamines as inducers of plant tolerance to stressors can be considered as an argument in favor of their adaptive value. Several studies showed an exogenous polyamine-attributed enhancement in the drought tolerance of common wheat. Thus, spraying wheat with putrescine and spermine enhanced plant tolerance to soil drought at the early stage of development, which was manifested as mitigation of growth suppression, abatement of water deficit (especially under the influence of putrescine) and a reduction in oxidative stress intensity [146]. In addition, the treatment of plants with both putrescine and spermine at concentrations of 1 and 5 mM contributed to the preservation of the pools of chlorophylls and carotenoids in leaves under stressful conditions. In the variants with polyamine treatment, a decline in the drought-induced increase in the proline content in leaves was noted. At the same time, both polyamines caused the accumulation of sugars in the leaves of treated plants and contributed to the preservation of the flavonoid pool. Thus, foliar treatment with polyamines, especially putrescine, had a positive effect on the osmoprotective and antioxidant systems [146].

H.T. Ebeed [147] revealed that both 10-hour soaking of wheat grains and plant spraying with polyamines improved growth under drought. In the variants with polyamine treatment, an enhanced accumulation of osmolytes, an improvement in the water status and an increased expression of genes for syntheses of endogenous polyamines were observed. In particular, it was found that the treatment with spermine and a putrescine-spermine mixture could upregulate endogenous polyamine synthesis pathways, enhancing the expression of genes for the key enzyme of polyamine synthesis, arginine decarboxylase [147].

There is information on the augmentation of drought tolerance of wheat plants due to foliar treatment with polyamines both in the vegetative and generative phases of development. For example, spraying wheat plants with 0.1 mM putrescine intensified photosynthesis during anthesis, mitigated growth suppression and boosted the grain productivity of plants [148]. At the same time, a decrease in the contents of proline and other amino acids was noted in leaves.

γ-Aminobutyric acid

All three possible isomers of non-proteinogenic aminobutyric acid (α, β, and γ) are found in plants [149]. Very little is known about the role of endogenous α-aminobutyric acid, while the other two isomers, β- and γ-aminobutyric acids, are important components of adaptive responses in plants [150]. However, the amount of β-aminobutyric acid in plants (data for Arabidopsis) is 1,200-fold lower than the content of γ-aminobutyric acid [151]. In this regard, γ-aminobutyric acid (GABA) is most intensively studied as a plant stress metabolite.

GABA is formed mainly in two ways in plants [152] (Fig. 4). The main pathway is the glutamate decarboxylation reaction catalyzed by cytoplasmic glutamate decarboxylase [153]. Alternatively, GABA can be produced in peroxisomes from putrescine after its oxidation by diamine oxidase [25]. As noted above, this reaction is the main reaction of putrescine catabolism. It generates H_2O_2, NH_3, and 4-aminobutanal (Fig. 4). The latter undergoes cyclization to form Δ^1-pyrroline, which is converted to GABA by pyrroline dehydrogenase [154]. It is also known that 4-aminobutanal can be produced from spermidine by polyamine oxidase, making GABA synthesis from spermidine a plausible pathway [155].

Theoretical studies also indicate that GABA can be synthesized from proline [86]. As already noted, it is believed that hydroxyl radical neutralization by proline leads to Δ^1-pyrroline formation. The latter is a substrate for pyrroline dehydrogenase, which converts it into GABA [156] (Fig. 4). Computer modeling, as well as confirmed events of enhanced generation of hydroxyl radical and formation of proline and GABA in plants under stressful conditions, give grounds to assume that this pathway is real. However, at present, there is no direct experimental evidence that plant GABA is formed as a result of oxidative transformations of proline.

GABA is catabolized in mitochondria, where it is transported by GABA permease. GABA transaminase converts GABA into succinate semialdehyde, which is converted into succinate by NAD^+-dependent succinate semialdehyde dehydrogenase, with concurrent release of NADH [153, 154]. Succinate can be metabolized in the tricarboxylic acid cycle and converted into glutamate via α-ketoglutarate.

GABA content in plants is tens μmol/g of fresh matter [157, 158], significantly exceeding contents of other nitrogen-containing stress metabolites, including proline and polyamines. Various stressors can significantly increase GABA levels in plants [155]. Such a phenomenon was observed under drought in different species, in particular, Arabidopsis, common bean, soybean, sesame, and tomato [158, 159]. In wheat seedlings of different cultivars, an enhanced expression of the *GAD* gene and increased GABA content were noted during germination in media containing osmotically active substances – sorbitol and NaCl [160]. At the same time, there was a strong positive correlation between GABA synthesis and seed germination under stressful conditions. An increase in the GABA content in

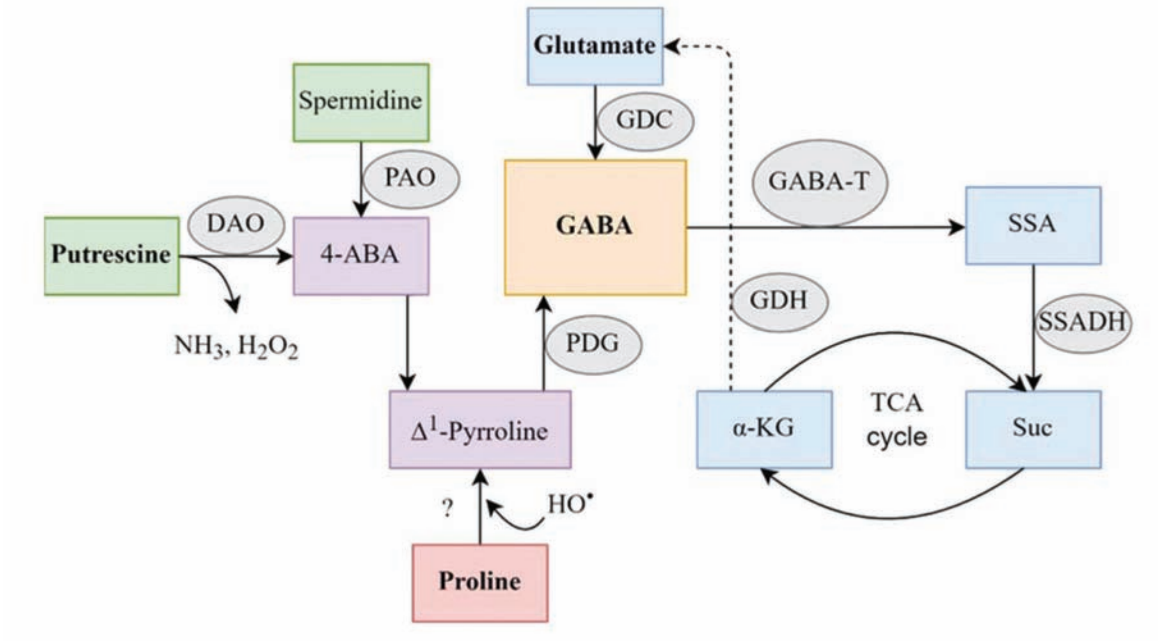

Fig. 4. Synthesis and catabolism of γ-aminobutyric acid in plants. GABA – γ-aminobutyric acid; GDC – glutamate decarboxylase; GABA-T – GABA-transaminase; CCA – succinic semialdehyde; SSADH – succinate semialdehyde dehydrogenase; Suc – succinate; α-KG – α-ketoglutarate; DAO – diamine oxidase; 4-ABA – 4-aminobutanal; PAO – polyamine oxidase; PDG – pyrroline dehydrogenase, TCA – tricarboxylic acid. GDH – glutamate dehydrogenase. Other explanations in the text

wheat in response to drought was also noted at later stages of development [161].

The stress-induced enhancement in GABA synthesis likely reflects glutamate decarboxylase activation caused by decreased cytoplasmic pH [153]. Another mechanism for increasing glutamate decarboxylase activity is associated with the binding of the Ca/calmodulin complex to the enzyme, which in turn is due to augmented calcium ingress into the cytosol under stressful conditions [25].

At the same time, it is likely that GABA in drought-stressed plants is formed not only from glutamate by glutamate decarboxylase. For example, in a study on wheat seedlings exposed to 20% PEG 6000, a significant increase in the GABA content was recorded, but with a slight increase in the glutamate decarboxylase activity [162]. This may indicate that different enzymes control GABA formation. In clover, the osmotic stress-triggered increase in GABA content was prevented not only by a glutamate decarboxylase inhibitor (4N), but also partially by a diamine oxidase inhibitor (aminoguanidine) [163]. This suggests that a part of the GABA pool can be formed in the course of putrescine catabolism during osmotic stress.

The mechanisms of the stress-protective effects of GABA can be different. GABA is thought to be an important component of the free amino acid storage system in plants, maintaining the carbon and nitrogen metabolisms, which are necessary for plant growth, and this is consistent with high levels of GABA in plant tissues [164]. GABA plays an important role in maintaining pools of reducing equivalents, which are important for both energy balance and cellular redox homeostasis under stressful conditions [155, 165, 166]. Under drought and other stresses associated with dehydration of plant tissues, GABA, along with proline, can make a major contribution to a rise in osmotic pressure in cells [164].

However, GABA effects are probably not limited to its direct protective action as an osmolyte and stress metabolite. There is experimental evidence of GABA involvement in the signaling network functioning in plant cells, including the regulation of ROS formation and neutralization as well as the state of calcium channels [167-169]. Mechanisms of GABA influence on these processes are very complex, so discussion of the mechanisms is beyond the scope of this review, but they are discussed in several recently published reviews [155, 170, 171].

Many stress-protective effects of GABA, in particular, antioxidant system activation, are apparently associated with GABA participation in signal transmissions. It is known that GABA exerts antioxidant and antiradical activities [172]. Its antioxidant effects may also be related to maintaining pools of reducing agents due to GABA shunt activation [173]. At the same time, exogenous GABA-induced activation of antioxidant enzymes in roots of wheat seedlings (the development of heat resistance as well) was eliminated when seedlings were treated with antioxidants and calcium antagonists [169]. These findings indicate the involvement of signaling mediators in stress-protective effects of GABA. In general, the ability of GABA to increase the activities of antioxidant enzymes and enhance the expression of their genes was demonstrated in different species and under various stressors [174, 175].

Other physiological effects of GABA include its ability to maintain the functioning of the photosynthetic apparatus under stressful conditions [175], influence the accumulation of ions in cells, and improve tissue hydration under dehydration-inducing stresses [176]. The latter effect may be attributed to GABA impact on the contents of major osmolytes (proline and sugars) under drought, which was recorded in experiments on different cereal species, including wheat, triticale, and creeping bentgrass (*Agrostis stolonifera* L.) [177-179].

In the context of stress-protective effects of GABA under drought, recent data on its effect on stomata deserve special attention. Using A. thaliana as a model, the researchers showed that GABA formation in guard cells was a necessary and sufficient effect to reduce stomatal opening and transpiration water loss [158]. An increase in the endogenous GABA level in leaves was recorded after 3- to 7-day drought. GABA modulation of the stomatal state was reported for many plant species, including dicotyledonous and monocotyledonous crops [158]. The main mechanism of such effects is considered to be GABA-signaled downregulation of the anion transporter localized in the tonoplast of stomatal guard cells.

Despite intensive research into the stress-protective and signaling functions of GABA in plants, there is still little information on its role in wheat adaptation to drought. When comparing the effects of osmotic and salt stresses on five wheat accessions, the authors revealed that the most significant enhancement in the expression of the glutamine decarboxylase gene was noticeable in the most tolerant cultivar; it also accumulated the greatest amount of GABA [160]. A study of amino acid compositions of two durum wheat cultivars differing in drought tolerance also showed that the constitutive and stress-induced GABA contents were higher in the tolerant one [180]. At the same time, Guo et al. [71] reported that after a long-term (15-day) drought, the GABA content in the tolerant cultivar was lower than in the susceptible one. It is possible that stress-induced changes in GABA levels in different cultivars have different time profiles during drought and also depend on drought intensity. It is also plausible that the GABA pool can be partially used to synthesize other osmolytes, for example, proline, during drought. These issues remain unexplored so far.

Effects of exogenous GABA on drought tolerance and protective systems were investigated in several studies on wheat and some other cereals. It was shown that an exogenous GABA-caused increase in wheat tolerance to drought was accompanied by an increase in the total content of phenolic acids due to the activation of phenolic ammonia lyase, cinnamic acid 4-hydroxylase and some other enzymes involved in syntheses of secondary metabolites [181].

When comparing the effects of exogenous GABA on the tolerance of two wheat cultivars to PEG 6000-induced osmotic stress, the researchers observed a more pronounced positive effect of GABA on the growth parameters of the susceptible cultivar [178]. GABA treatment reduced the contents of oxidative stress markers (hydrogen peroxide and MDA), stabilized the activities of antioxidant enzymes (SOD and guaiacol peroxidase) and prevented a decrease in the content of flavonoid compounds in both cultivars. It is noteworthy that GABA enhanced stress-induced proline accumulation in the susceptible cultivar but dampened it in the tolerant one [178]. In triticale plants at the early stage of development, GABA treatment increased the tolerance to drought, salt stress, and their combination. Concomitantly, like in wheat seedlings, GABA mitigated oxidative damage, stabilized or increased the activities of key antioxidant enzymes and increased the contents of proline, sugars and phenolic compounds [179].

Interesting effects of GABA were recently discovered upon wheat seed priming. It was shown that the positive effect of seed soaking in a low (1 μM) concentration of GABA on plant drought tolerance persisted 6 weeks after treatment. GABA priming of seeds significantly enhanced antioxidant protection mechanisms, accumulation of proline, phenolic compounds, and endogenous GABA in leaves, thereby stabilizing the photosynthetic apparatus functioning and growth of wheat subjected to drought [182].

Metabolic and functional relationships between nitrogen-containing stress metabolites

Proline, polyamines, glutamate, and GABA in plant cells are intertwined in metabolic cycles (Fig. 5). Glutamate is the major substrate for syntheses of proline and GABA [183]. At the same time, glutamate is formed in proline catabolism as well as from α-ketoglutarate, the pool of which is driven by the GABA shunt. During the catabolism of putrescine and other polyamines, 4-aminobutanal, which can be converted into GABA via Δ^1-pyrroline, is formed.

It should be noted that glutamate is considered as a common substrate in biosyntheses of not only proline and GABA but also polyamines, although it is not their immediate precursor [170, 184]. It is assumed that glutamate can be converted to putrescine via N-acetylglutamate and N-acetylornithine [185]. On the other hand, putrescine is synthesized not only from arginine (this pathway is recognized as the main one) but also from ornithine. The latter is also the parent substance for the synthesis of P5C, which can be converted to both proline and glutamate. Thus, syntheses of putrescine, proline, glutamate, and GABA (indirectly) can compete for ornithine pool [183]. In addition, along with glutamate, putrescine and presumably proline (because of its conversion to Δ^1-pyrroline during non-enzymatic interaction with ROS) can be substrates for GABA synthesis [86]. Contents of proline, polyamines, glutamate and GABA in plant cells are quite comparable, which allows us to consider the above-discussed metabolic relations as real. However, the regulation of these transformations remains poorly studied,

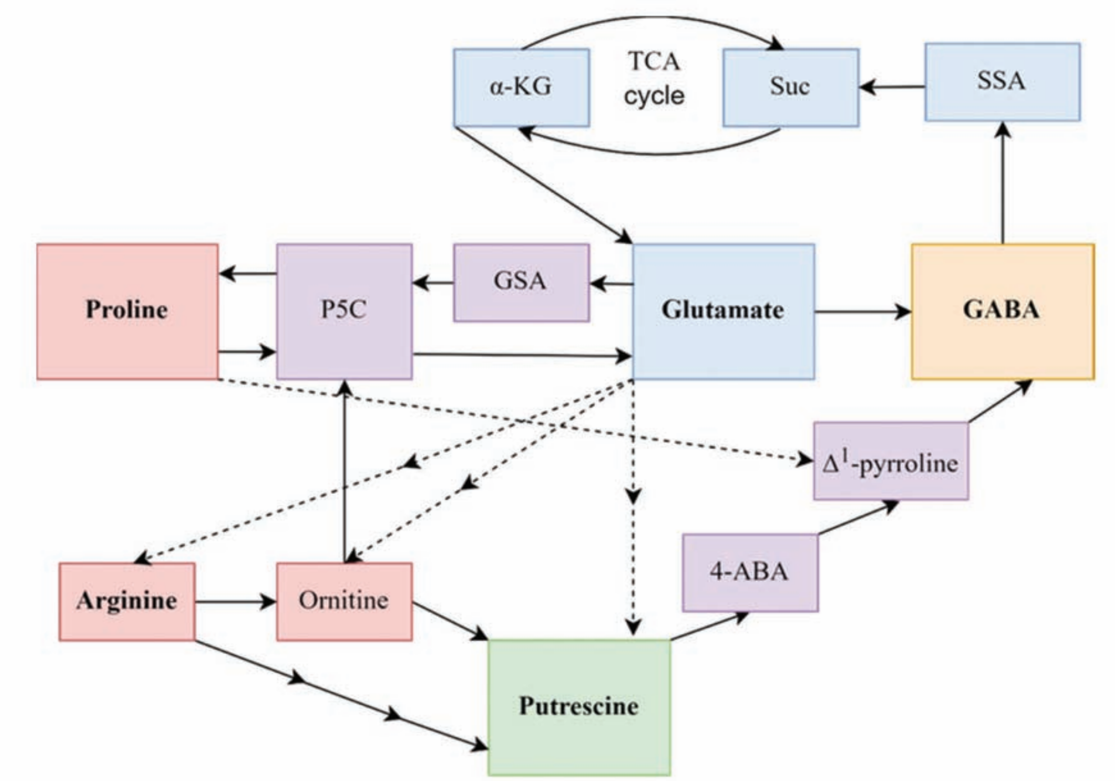

Fig. 5. Metabolic relations between nitrogen-containing stress-protective compounds. GABA – γ-aminobutyric acid; SSA – succinic semialdehyde; Suc – succinate; TCA – tricarboxylic acid; α-KG – α-ketoglutarate; GSA – glutamate semialdehyde; P5C – Δ^1-pyrroline carboxylate; 4-ABA – 4-aminobutanal. Intermittent arrows indicate transformations that occur at several stages. Dotted arrows indicate links that do not have unambiguous experimental evidence. Other explanations are given in the text

and it is usually very difficult to give unambiguous answers to questions regarding triggers of changing one substrate to another in metabolic pathways.

The interactions of nitrogen-containing compounds as metabolites are discussed above. However, as already noted, all these compounds can perform regulatory functions to various extents. In this context, the reciprocal influence on their syntheses (for example, when a plant receives one of them exogenously) is complex and not always predictable on the basis of the known transformation pathways for these compounds. For example, exogenous polyamines can both enhance and dampen proline synthesis in plants. Such effects are especially ambiguous when these compounds are investigated in combination with stressors. Doneva et al. [138] showed that treatment of young wheat plants with 0.5 mM putrescine did not affect the proline content under normal conditions but significantly reduced its stress-induced accumulation, both in drought-tolerant and susceptible cultivars. On the other hand, there was a direct relationship between exogenous putrescine catabolism and proline accumulation in drought-stressed wheat, with a Cu-dependent amine oxidase inhibitor suppressing proline accumulation [183]. H.T. Ebeed [147] experimented on young wheat plants and observed an increase in the proline content in aerial parts under drought in variants with pre-sowing treatment of seeds with putrescine or (to a lesser extent) spermine. At the same time, foliar treatment with putrescine, on the contrary, reduced drought-caused proline accumulation, while spermine treatment did not affect it. Similar results were obtained when the effects of polyamines on the proline content were studied in etiolated seedlings subjected to PEG 6000-induced osmotic stress and in young green plants exposed to soil drought. Exogenous putrescine, when supplied through roots, increased the proline content in stressed shoots [186], while foliar treatment with putrescine made a soil drought-caused rise in the proline content significantly less pronounced [146]. It was also shown that exogenous spermine had no noticeable effect on the proline content under stressful conditions both in shoots of etiolated seedlings and in green plants [146, 186]. At the same time, Hasan et al. [187] reported in their review that a spermine-induced enhancement in drought tolerance from plants of different taxonomic groups was accompanied by an increase in the proline content. In addition, it was shown that the improved germination of spermidine-treated wheat seeds under osmotic stress was concomitant with an increase in the proline content in shoots [188].

In general, the presented data seem to be rather contradictory. At the same time, they give reason to believe that the effects of different polyamines may be specific. Apparently, they may depend on the species and varietal characteristics of plants, the developmental stage, the dosage of exogenous polyamines, and the way of their entry into plants. No increase or even a decrease in proline levels in polyamine-treated plants, which have been observed in some of the studies mentioned above, gives reason to believe that the conversion of exogenous polyamines into proline in the GABA-glutamine metabolic pathway (Fig. 5) is not their obvious effect. On the other hand, it was shown that drought-adapting wheat plants can use proline pool for polyamine syntheses [138]. Thus, it is apparent that the adaptive responses of plants can include mutual transformations of these compounds.

An important feature of polyamines consists in the fact that they act not only as metabolites but also as regulatory molecules, in particular, sources of key signaling mediators – ROS and nitric oxide [117]. As already noted, we showed that, for example, exogenous putrescine was capable of ROS-mediated activation of the enzymatic antioxidant system [134], which may be important for the adaptation to different stressors, including drought. In addition, polyamines can induce sugar accumulation and stabilize flavonoid pool, and flavonoids are important antioxidants [146, 186]. In other words, in addition to participating in the direct formation of important nitrogen-containing metabolites (in particular proline) and direct protective effects on cell membranes and biomacromolecules, polyamines are involved in the complex signaling network that functions during the activation of adaptive responses of plants to drought [117].

Exogenous GABA, like polyamines, can replenish glutamate pool through the GABA shunt, which, in turn, can be used to synthesize proline. The probability of such events seems quite high, since several studies demonstrated that exogenous GABA in rather high concentrations (usually from 0.1 to 2 mM) enhanced drought tolerance in plants [189], and even higher concentrations (up to 50 mM) were required in some studies [168]. A noticeable increase in the proline content in shoots of etiolated triticale seedlings was noted under the influence of 0.5 mM GABA both without stress and under PEG

6000-simulated drought [179]. On the other hand, in etiolated wheat seedlings subjected to osmotic stress, GABA increased the proline content only in the drought-susceptible cultivar, while in the tolerant cultivar, stress-induced proline accumulation was subdued under the influence of GABA [178]. It is difficult to give an unambiguous explanation for such varietal peculiarities of the responses to exogenous GABA. As already noted, increased proline content is a response of plants to rather strong stressors [190]. It is possible that a moderate stress increases proline content as an adaptive response, especially in susceptible cultivars, and GABA promotes this reaction, acting as a source of substrate for proline formation. On the other hand, GABA stabilized the key antioxidant enzyme (SOD) activity and sugar content and propelled the accumulation of secondary metabolites under stressful conditions in both susceptible and tolerant wheat cultivars [178]. Such effects may result from its positive influence on energy processes, maintenance of redox homeostasis (through activation of the GABA shunt) and inclusion in the cellular signaling network. It was shown that a GABA-triggered increase in the activities of antioxidant enzymes in roots of wheat seedlings was abolished by antioxidants and calcium antagonists, indicating that ROS and cytosolic calcium participate in these reactions [179].

Interesting phenomena were observed when individual and combined effects of exogenous GABA and proline on oilseed rape plants exposed to drought were studied [191]. At the initial stage, both compounds reduced drought-induced proline accumulation. At the same time, at the late stage, GABA and proline, when acting separately, increased proline accumulation; however, in the experiment with their combination, the proline content was significantly lower than in all other variants, except for the control. It is difficult to give a convincing explanation for such phenomena at the moment. However, there is reason to believe that some of the effects of GABA are associated with its action as a metabolite, which is converted into other nitrogen-containing compounds with stress-protective activities (primarily into proline), while other effects are attributed to signaling network activation. The role of GABA in the signaling network remains a complex and poorly understood issue that is beyond the scope of this review.

Conclusion. Most plant stress metabolites are multifunctional compounds necessary for plant adaptation to drought. All of them function as osmolytes to varying degrees. Apparently, soluble carbohydrates make the greatest contribution to the maintenance of osmotic pressure in drought-stressed cells, since these compounds accumulate in cereal organs in large amounts, significantly exceeding, for example, proline accumulation. In numerous studies, there was a strong correlation between sugar content and drought tolerance of wheat genotypes. Contributions of proline and especially other amino acids, as well as polyamines, betaines and some other nitrogen-containing metabolites to osmoregulation, are probably somewhat smaller. At the same time, all these compounds have a colligative effect on osmotic pressure in plant cells and its increase as an adaptive response to drought.

Antioxidant activity is an important function of most stress metabolites. Many of them significantly complement the enzymatic antioxidant system, which is not able to neutralize most radical ROS. It is known that soluble carbohydrates such as trehalose, raffinose, fructans, sobitol, etc. are highly efficient in binding hydroxyl radicals. *In vitro* experiments showed that the hydroxyl radical could be successfully neutralized by proline; hence, through this lens, proline has been considered recently not only as an osmoprotector but also as an important antioxidant. Polyamines, especially spermidine and spermine, are also recognized as active scavengers of radical ROS. Such properties of polyamines are partly due to easy auto- and enzymatic (by di- and polyamine oxidases) oxidation of amino groups. On the other hand, these processes lead to hydrogen peroxide generation, and hydrogen peroxide can both perform signaling functions (see below) and cause oxidative damage if not neutralized by antioxidant enzymes. In this regard, polyamines are also seen as pro-oxidant agents in some cases. The antioxidant capacity of GABA is less studied; nevertheless, its potent ability to interact with ROS was shown *in vitro*.

Other important functions of stress metabolites are membrane protection and anti-denaturation. Such properties are intrinsic to soluble carbohydrates; for example, trehalose, whose hydroxyl groups form hydrogen bonds with polar protein groups and membrane lipid phosphates during cell dehydration. Raffinose molecules prevent cell membrane fusion upon severe dehydration. Proline plays a crucial role in stabilizing proteins during stress-related impairment of cellular homeostasis. It can have a direct stabilizing effect on protein molecules, acting as a chemical chaperone. Upon severe dehydration of cells, proline can stabilize cellular structure through hydrophilic interactions and hydrogen bonds. Along with proline, polyamines, which are capable of binding to phospholipid heads on membranes, have stabilizing effects on proteins, nucleic acids and membrane structures.

Despite the ability of most stress metabolites to exert direct protective effects in various ways, their signaling and regulatory functions appear to be most important. However, these functions remain poorly studied. Nevertheless, data on the roles of most of the compounds mentioned in this review in cellular signaling pathways are rapidly accumulating. In particular, it is known that even the simplest carbohydrate, glucose, affects plant ontogenesis through hexokinase-dependent pathways, can modulate the enzymatic antioxidant system functioning and when supplemented exogenously, increase plant tolerance to abiotic stresses [46, 47].

The signaling functions of polyamines can be considered fully proven, at least in relation to their ability to transformations accompanied by ROS and NO generation. Recently, the signaling functions of GABA and proline have been intensively studied. It is important that these nitrogen-containing metabolites are easily converted into other compounds and reciprocally influence the synthesis of each other. In this regard, identification of potential receptors for intermediate products of GABA or proline metabolism, such as P5C, is of paramount interest, since these molecules are usually detected in much lower concentrations, which makes them potentially more suitable for roles of signaling molecules [96]. Switching of low-molecular compounds from one role to another, for example, from the role of metabolites *per se* to the role of specific protective compounds and especially to the role of components of cellular signaling pathways, remains a mystery. One can only hope that computer simulations, omics and genome editing methods will allow consolidating extensive and various information on the contributions of these molecules to drought tolerance mechanisms in plants [28, 96].

It can be stated that by now, as exemplified on one of the most economically important cereal species, *Triticum aestivum*, abundant useful knowledge about stress metabolites has been gained, and it can be used in practice to enhance drought tolerance. Levels of soluble carbohydrates, proline and, to some extent, other low-molecular nitrogen-containing compounds can be used in screening for drought-tolerant genotypes. Genetic modifications that enhance osmolyte accumulation (e.g., proline) in plants not only improve drought tolerance but also modulate multiple protective systems, including tolerance to oxidative stress. However, transgenic plants with such features were not tested in the field, which so far limits forecasts for their widespread use [35]. Nevertheless, exogenous polyamines and GABA, at least under controlled conditions, are highly efficient in inducing drought tolerance in wheat and other cereals. These findings support the practical relevance of further research into stress metabolites.

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СТРЕСОВІ МЕТАБОЛІТИ У ПШЕНИЦІ: РОЛЬ В АДАПТАЦІЇ ДО ПОСУХИ

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Посуха є одним з основних чинників, що обмежують сільськогосподарське виробництво та призводять до втрат врожаю. Пшениця, яка є джерелом їжі для більш ніж половини населення світу – дуже чутливий до посухи вид рослин. У зв’язку з цим особливий практичний інтерес становить дослідження її видоспецифічних стресових метаболітів та фізіологічних і біохімічних механізмів посухостійкості. У цьому огляді всебічно розглядається роль розчинних вуглеводів, проліну, поліамінів та ГАМК, а також їхня функціональна взаємодія при адаптації пшениці та інших видів рослин до посухи.

Ключові слова: посуха, *Triticum aestivum*, стресові метаболіти, розчинні вуглеводи, пролін, поліаміни, гамма-аміномасляна кислота, осмопротектори, антиоксиданти, клітинний сигналінг.

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