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Review article

The Role of Exogenous Glutamine as a Regulator of Gene Expression under Stress Conditions in Plants

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Abstract

Amino acids, which are necessary for the synthesis of proteins as well as some other nitrogenous components in organisms, are effective for the synthesis and in the activities of some enzymes and of gene expression. Stress tolerance is provided by different mechanisms, especially with the accumulation of some specific amino acids under stress conditions in plants. Although the role of some amino acids such as proline under stress conditions has been demonstrated by many studies, the mission of some other amino acids under stress conditions has not yet been fully elucidated. Like other amino acids in organisms, glutamine is involved in the synthesis of nitrogenous compounds such as amino acids and nucleotides. The functions of glutamine in plants, which are known to be involved in signal transmission in humans, yeast, and bacteria, are not yet fully known. For this reason, various studies conducted in recent years have focused on elucidating the role of glutamine in signal transduction pathways under stress conditions. It was revealed by transcriptome analyses that exogenous glutamine applications support growth and development in some plants by inducing the expression of several genes involved in metabolism, transport, signal transduction, and stress response. It was identified that these genes synthesize transcription factors that activate the genes involved in nitrogen metabolism or stress response. The induction of these transcription factor genes by glutamine supports the idea that it functions as a signaling molecule regulating gene expression in plants. In this review, research studies investigating the role of glutamine, especially under stress conditions, were examined, to create a resource for researchers of future studies on this topic.

Keywords: Abiotic Stress, Exogenous application, Glutamine, Plant, Signal Transduction.

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INTRODUCTION

The increasing effect of abiotic stress factors, which cause a decrease in plant yield and quality, especially global climate change, makes it difficult to meet the nutritional needs of the rapidly increasing human population. This situation highlights the need to develop new strategies to cope with environmental stresses. Thus, the use of amino acids in plants to cope with abiotic stresses is one of the topics that has been widely researched recently (Haghighia et al. 2020, Batista-Silva et al. 2019).

Amino acids, which are the monomers that makeup proteins, are the precursors of nitrogenous organic compounds, such as nucleotides. Amino acids play different roles in plant metabolism in response to abiotic stress. During abiotic stress, they act as substrates for mitochondrial ATP production and as osmoprotectants in maintaining intracellular osmotic pressure, and are precursors for the synthesis of stress-induced proteins and secondary metabolites. Another function that has been investigated in recent years is their role in the formation of the stress response in the intracellular signal transmission network (Heinemann and Hildebrandt 2021). The accumulation of different amino acids, especially proline, under stress conditions in plants is associated with functions such as the regulation of ion transport, the regulation of stomatal movements, heavy metal detoxification, and being an osmolyte (Rai, 2002). Signal transmission through amino acids in plants is a topic that has not yet been fully clarified (Heinemann and Hildebrandt 2021). Although the role of glutamine in stress response formation, like many other proteinogenic amino acids in amino acid metabolism that changes under stress conditions, has not yet been fully elucidated, some existing research studies have showed that it induces transcription factors that activate the genes responsible for the expression of proteins that will ensure plant survival under abiotic stress conditions (Kan et al. 2015; Miranda et al. 2017).

The Metabolic Functions of Glutamine in Plants

In recent years, functional genomic and omic studies conducted to evaluate metabolites obtained from cells or tissues of plants exposed to stress and their quantitative analyses are useful methods for determining the responses of plants to stress conditions (Kim et al. 2007; Nam et al 2015)

Specific metabolites are synthesized by molecular and physiological regulations in the formation of stress tolerance in plants. The physiological response created by regulating the level of these synthesized metabolites by both transcriptional and post-translational mechanisms at the proteome level provides tolerance to stress conditions in the plant. Although amino acids synthesized under stress conditions in plants have been investigated in existing research, there are only a few studies on the identification of genes producing these metabolites and on amino acid catabolism. Glutamine, one of the specific metabolites synthesized by plants to cope with abiotic stresses, is the primary assimilation product by which inorganic nitrogen metabolizes in plants and is a precursor molecule for the formation of other amino acids (Coruzzi 2003; Hildebrandt et al. 2015; Muthuramalingam et al. 2020).

Nitrogen, which is a key element for the growth and development of plants as well as of other living organisms, is found in the structure of organic compounds such as protein and nucleic acid. Plants can obtain the ammonium necessary for the synthesis of glutamine, one of the proteinogenic amino acids, which are the building blocks of proteins, directly from the soil or by reducing nitrate. Ammonium can be incorporated into glutamine and glutamate via a cycle involving glutamine synthetase (GS) or glutamine oxoglutarate aminotransferase enzymes in plants (Tabuchi et al. 2007). GS enzymes are classified into three morphological categories, namely GS1, GS2, and GS3 (Woods and Reid, 1993). An increase in GS activity occurred in rice under salt stress (Yan et al., 2005) and low-temperature conditions (Lu et al., 2005), which increased the plant's tolerance to salt (Hoshida et al., 2000). In addition, the change in GS activity could be used in the selection of drought-tolerant cultivars (Singh and Ghosh, 2013). Glutamine, which functions as a key nitrogen source for plant growth and development, is one of the most abundant free amino acids in plants (Muthuramalingam et al. 2020). This amino acid, which is highly functional in plant growth and for metabolism under normal conditions, is also involved in the compartmentation and development of the ROS scavenging system, osmolyte biosynthesis, and the induction of transcription factors under stress conditions (Kan et al. 2015, Nam et al. 2015). The importance of glutamine in plant growth and development as a metabolite that regulates the assimilation of inorganic nitrogen in metabolism and its distribution under different physiological conditions is indisputable. In addition, the biosynthesis of protein and cytokinins, which are the main metabolic events in plants, are among other key roles of glutamine. In addition to these, especially in recent years, studies that determined that glutamine recognizes the signals from genes and receptors that enable the sensation of stimuli from the environment and those that specifically highlighted that it regulates the transcription factors related to abiotic stress tolerance have strengthened the idea that it also takes part in intracellular signal transmission and stress response formation (Tabuchi et al. 2007, Kamada-Nobusada et al. 2013; Kan et al. 2015, Nam et al 2015).

The Effect of Exogenous Glutamine Applications on Plants

Exogenous amino acid applications affect plant growth and development through the regulation of physiological activities in plants (Noroozlo et al. 2019, Khan et al. 2012). Amino acids have a stimulating effect on growth and yield (Sadak et al. 2015). The exogenous foliar application of an amino acid mixture positively affected the plant growth and fruit quality of a grapevine (Khan et al. 2012). Similarly, it has been shown that external amino acid applications in lettuce (Noroozlo et al 2019) and in potatoes (El-Zohiri et al. 2009) have growth- and development-promoting effects. As with other amino acids, glutamine has a positive effect on productivity in many crop plants. A foliar glutamine application to the leaves of onions had a positive effect on their plant growth parameters such as plant height, the number of leaves, and bulb length. The applied glutamine also increased the yield and quality of the onions. The glutamine application had a positive effect on the biochemical parameters as well as on the vegetative growth parameters and increased the total soluble sugar, phenol, and free amino acid content, sulfur compounds, and total photosynthetic pigment content in the leaves (Amin et al. 2011). Similarly to the results found in the study on the onion, a 200 mg/l glutamine application had a positive effect on the growth and yield of Vicia faba (Rashad et al. 2003). A foliar glutamine application in lettuce caused an enhancement in root and shoot growth, in the pigment in leaves, and in vitamin C content (Noroozlo et al 2019). The use of glutamine with proline in rice grown under in vitro culture conditions promoted callus and shoot formation (Pawar et al. 2015). It was identified that an exogenous glutamine application had a positive effect on plant regeneration in cucumbers (Vasudevan et al., 2004) and in bananas (Husin et al., 2014), and increased the biomass of arabidopsis plants (Forsum et al., 2008) and rice (Kan et al., 2015). On the other hand, other studies on arabidopsis plants (Kim et al., 2010) and on rice plants (Kan et al., 2015) revealed that it had an inhibitory effect on root elongation. Considering that this occurrence is dependent on the dose and genotype, it is thought that elucidating the molecular mechanisms of the changes caused by exogenous glutamine applications in plants is important for future applications. Compared to conventional fertilizers, providing nitrogen from amino acids, which is an organic source, is beneficial in maintaining the pH balance of both the plant and its soil. Thus, exogenous amino acid applications for plant health and nutrition are invaluable in terms of sustainable agriculture and environmental protection (Dellero, 2020).

The Effect of Exogenous Glutamine Applications on Plants Under Abiotic Stress Conditions

Amino acids have a stimulating effect on growth and yield under abiotic stress conditions as well as under normal conditions (Sadak et al. 2015). A high level of amino acid mixture (1500 mg/l) had an alleviating effect on all the morphological, biochemical, and genomic harmful effects of salt stress on Vicia faba (Sadak et al. 2015).

In a study on onions, glutamine treatment significantly increased germination and seedling growth of the allium cepa under both normal and salt stress conditions. In salt stress conditions, a glutamine application increased root length and plant fresh weight, and chromosomal aberrations caused by salt stress were greatly reduced (Çavuşoğlu et al. 2020). In both non-stress and salt stress conditions, an external glutamine application had a positive effect on plant growth in sugar cane plants. In addition, it increased the plant's tolerance to salt stress with its regulatory effect on antioxidant enzyme activities (Patade et al. 2014). GS activity, which is responsible for glutamine synthesis, increased in potato tubers growing under salt and drought stress (Teixeira and Pereira 2007). A study conducted on transgenic tobacco showed that glutamine was the main amino acid involved in the synthesis of proline, which is one of the most effective amino acids regarding tolerance to stress conditions (Brugiere et al. 1999).

Some Molecular Mechanisms Involved in the Formation of Stress Response Mediated by Glutamine in Plants

Several amino acids, including glutamine, act as stress biomarkers in the amino acid biosynthesis pathway, which is one of the few metabolic pathways that play a key role in the response to various abiotic and biotic stresses in different plants. It has been demonstrated by transcriptome analyses that amino acid signaling pathways interact with biotic and abiotic signaling networks in plants. The activation of glutamine under different stress conditions is thought to play a key role in stress adaptation (Ma et al. 2013; Kan et al. 2017).

Glutamine acts on different cellular functions through the activation of various transcription factors in mammalian cells. Among these transcription factors are the key genes involved in stress response formation (Brasse-Lagnel et al. 2009). In plants, the control of gene expression by glutamine, the primary metabolite of nitrogen assimilation from inorganic nitrogen sources, is not yet fully known. However, there are some recent studies on this subject.

Glutamine effectively promoted growth in rice seedlings and the amount of glutamine rapidly increased in glutamine-pretreated rice roots. Transcriptomic analyses revealed that glutamine induced the expression of at least 35 genes responsible for the regulation of nitrogen metabolism, signal transduction, and stress response formation in a short time. The expression of DREB1A, IRO2, and NAC5 transcription factor genes, which are involved in the formation of the stress response, is rapidly induced by glutamine. In particular, the induction of transcription factor genes suggests that glutamine may interact with other signal transduction pathways to regulate plant responses under stress conditions (Kan et al. 2015).

The response to stress conditions in plants occurs by activating signal transduction pathways involving various protein kinases. In this process, stress-related genes are induced, resulting in stress response formation and adaptation. Calcineurin B-like protein-interacting protein kinases are involved in stress conditions and in the calcium-mediated signal transduction mechanism, which is a universal intracellular messenger. Glutamine induces the expression of some kinases involved in different signal transduction pathways and transcription factors. Of these, CIPK 14 provides a remarkable response to biotic and abiotic stresses (Xiong and Yang 2003; Xiang et al. 2007; Kan et al. 2015).

The activation of the salt overly sensitive pathway that removes salt from plant cells is mediated by calcium-sensitive calcineurin B-like (CBL) proteins, and CBL interacting protein kinases (CIPKs). An increase in salt concentration in plant cells triggers a temporary increase in the calcium concentration in the cytoplasm. After perceiving this situation by the CBL, a CBL/CIPK complex is formed by interacting with the CIPKs. This complex results in the regulation of genes that control ion homeostasis in the cell. The CBL-CIPK network system is a specific and complex system that is involved in the response to salt stress as well as to other abiotic stresses. A hypothesis was proposed that glutamine may have a possible role in the activation of CBL/CIPK signaling pathways under salt stress in sorghum. Researchers reported that this hypothesis was supported by the fact that glutamine accumulation in sorghum roots treated with NH_4 was more pronounced under salt stress conditions (Miranda et al. 2017).

The intracellular increase of glutamine in rice activated some genes known to be associated with stress. One of the possible mechanisms of glutamine induction of stress-related genes such as DERB1A, IRO2, and HMA is thought to occur by increasing intracellular ROS production. Thus, glutamine, which supports plant growth and development under normal conditions, can also be evaluated as a molecule that reveals the plant's defense responses by interacting with signal transmission pathways under stress conditions (Kan et al. 2015).

In the computational metabolomic genome study conducted in *Oryza sativa*, five abiotic stress responsible (AbSR)–Gln metabolite-producing genes (GlnMPG) were identified under different abiotic stress conditions (Muthuramalingam et al. 2020).

There is a close relationship between nitrogen status and plant–pathogen interaction. A research study on *Arabidopsis* plants demonstrated that glutamine plays a critical role in the formation of disease resistance by moderating the cellular salicylic acid-associated redox status (Liu et al. 2010).

Conclusion

The identification of metabolites synthesized in plants against environmental stresses is the first step in understanding the signaling pathways and molecular mechanisms that mediate the synthesis of these metabolites under stress conditions. These studies will contribute to biotechnological applications made in plants in terms of the sustainability and safety of the world food supply in increasingly unfavorable environmental conditions.

In this review article, studies investigating the functions of glutamine under both normal and stress conditions were presented. In particular, existing research on the topic was identified by focusing on some molecular mechanisms mediated by glutamine, which was identified in several studies on the role of glutamine, a relatively new subject, in intracellular signal transmission and stress conditions.

It was shown in some studies that glutamine, which is a precursor molecule for the synthesis of nitrogenous compounds in all organisms, causes the expression of some stress-related genes by activating pathways that provide intracellular signal transmission under stress conditions. However, the role of glutamine in stress conditions has not yet been fully clarified. Therefore, genomic and transcriptomic studies in different plants will provide more information on this subject.

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