UP REGULATING THE FLAVONOID BIOSYNTHETIC PATHWAY OF SOYBEAN USING MYB TRANSCRIPTION FACTOR FOR ENHANCED PRODUCTION OF PHARMACOLOGICALLY IMPORTANT ANTIOXIDANTS

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EXECUTIVE SUMMARY

Soybean (*Glycine max* (L.) Merrill), a highly valuable legume in the Fabaceae family, is recognized for its rich nutritional content and therapeutic properties (Chen et al., 2012). It is a significant source of protein, carbohydrates, vitamins, and minerals (Liu, 1997; M., 2013). In its dry form, soybean consists of approximately 36% protein, 19% oil, and 35% carbohydrates, along with dietary fiber (Davis et al., 2005). It also contains essential bioactive compounds such as phytic acid, isoflavones, and flavonols. Flavonols, the most prevalent flavonoids in plants, commonly exist as mono-, di-, and triglycosides (Veitch and Grayer, 2008), with quercetin and rutin being the most notable. These compounds play a protective role against ultraviolet (UV) radiation (Lännenpää, 2014) and influence auxin signaling and transport (Peer and Murphy, 2007; Singh et al., 2021). In humans, flavonols contribute to antioxidant activity, cardiovascular and brain health, skin protection, anti-inflammatory responses, cancer prevention, gut health, diabetes management, and defense against age-related diseases (Luo et al., 2008; Misra et al., 2010; Salehi et al., 2020). Due to their health-promoting properties, there is growing scientific interest in increasing flavonol content in plants (Pandey et al., 2012; Lännenpää, 2014). With the global population expected to surpass 9 billion by 2050, there is an urgent need to develop crops with higher yields, improved quality, and lower input requirements, such as reduced irrigation and fertilizer use (Tilman et al., 2011). Since many people rely primarily on plant-based diets, micronutrient deficiencies remain a widespread issue, preventing populations from meeting recommended daily intake levels (Tucker, 2003). This highlights the importance of crop biofortification, which can align with consumer needs while improving health outcomes. Given that plants are a key dietary source of antioxidants, enhancing flavonol levels in soybeans could help mitigate oxidative stress-related diseases. Therefore, biofortified soybean presents a promising solution for addressing global nutritional challenges, improving public health, and supporting food security.

Environmental factors such as drought, heavy metals, herbicides, and salinity pose significant challenges to crop growth and productivity (Saravanan et al., 2023). These stressors hinder plant development, reduce yield, and ultimately compromise both quality and quantity, sometimes leading to plant death. Among these, soil salinity is a major abiotic stressor that severely affects agricultural land, limiting its usability. Exposure to high salt concentrations disrupts cell division and elongation in plants, leading to stunted growth (Chourasia et al., 2022). Soybean is one of the most widely cultivated crops worldwide and exhibits moderate salt tolerance, with an average threshold of approximately 5 dS m^{-1} (Rao and Reddy, 2010; Khomari et al., 2018). However, salinity stress can cause up to a 40% reduction in yield, depending on its severity (Hasanuzzaman et al., 2022). The adverse effects of salt stress on soybean include inhibited cell division and elongation, which ultimately result in reduced root length, fewer roots, decreased root biomass, and impaired nodule formation (Egamberdieva et al., 2017; Chourasia et al., 2022). These negative impacts not only lower crop yields but also lead to financial losses for farmers. Therefore, improving the salt tolerance of soybeans is essential for promoting sustainable agriculture and maintaining their economic viability.

Various scientific strategies are utilized to enhance the nutritional value and stress tolerance of soybeans, including breeding programs, agronomic advancements, and transgenic approaches. Among these, transgenic technology stands out due to its efficiency and precision, allowing for targeted genetic modifications that accelerate the development of soybean varieties with desirable traits. Several genes and transcription factors (TFs) have been identified and utilized in soybean transformation efforts. One such group, MYB (Myeloblastosis) proteins, comprises a diverse family of plant proteins characterized by a conserved DNA-binding region known as the MYB domain (Ambawat et al., 2013; He et al., 2020). The R2R3-type MYB proteins, featuring two MYB domain repeats, represent the most prevalent subfamily in plants. *Arabidopsis thaliana* MYB12 transcription factor (*AtMYB12*) belongs to R2R3-MYB family and serves as a key regulator of structural genes involved in flavonol biosynthesis within the phenylpropanoid pathway (Lepiniec et al., 2006). Previous research highlights that *AtMYB12* as a strong candidate for enhancing flavonol accumulation and improving salt tolerance in soybean. Therefore, the objective of this project is to overexpress *AtMYB12* to boost flavonol content and enhance soybean's resilience to salt stress.

The objective of the proposed project includes of cloning of *AtMYB12* along with seed specific promoter vicilin into plasmid pCAMBIA 1301 and mobilizing the recombinant vector into *Agrobacterium tumefaciens* strain EHA105, generation of transgenic soybean plants expressing *AtMYB12* using in-planta seed transformation technology, molecular confirmation of transgene integration using GUS assay, PCR, and Southern blotting, inheritance analysis of transgene in successive generations, quantification of flavonols in seeds of transformed soybean using HPLC, biochemical analysis and assessment on antioxidant potential of flavonols in seeds of transformed soybean, RT-qPCR analysis of structural genes involved in flavonols biosynthesis of transformed soybean, assessment on cytotoxic activity of flavonols extracted from transformed soybean seeds in selected cancer cell lines, and assessment of salt tolerance in *AtMYB12*-transformed soybean.

The *AtMYB12* transcription factor was cloned into pCAMBIA 1301 vector and then introduced into the soybean cultivar JS335 using *Agrobacterium tumefaciens* strain EHA105 to boost both flavonol content and salt tolerance. The potential transformants exhibited gus expression in their mature leaves, trifoliate leaves, and stems. PCR analysis confirmed the presence of *AtMYB12* by producing an amplicon of 786 bp. Compared to non-transformed plants, the transgenic seeds showed significantly higher levels of total phenolics, flavonoids, and antioxidant activity, with HPLC results indicating increases of 1.5-fold in rutin and 1.3-fold in quercetin. The successful integration of the *AtMYB12* transgene was also evident from the elevated expression of key flavonol biosynthetic genes during various stages of seed development. Furthermore, by overexpressing *AtMYB12*, the study aimed to enhance soybean's salt tolerance. Ten-day-old PCR-positive *AtMYB12* transgenic seedlings under salt stress displayed marked improvements in their morphological traits, photosynthetic pigments, and leaf relative water content when compared to non-transformed plants facing the same stress. Additionally, *AtMYB12* overexpression helped to mitigate membrane damage and increased enzymatic antioxidant activities, osmoprotectants, polyphenolics, and flavonoids. The overexpression of *AtMYB12* also upregulated genes related to flavonol, abscisic acid, and proline biosynthesis, as well as ion transport. These findings underscore *AtMYB12* potential in reducing salt stress and bolstering soybean resilience, making it a promising candidate for concurrently enhancing flavonol content and salt tolerance in soybean.

Publications from the project:

1. Saravanan, K., Vidya, N., Appunu,C., Gurusaravanan, P., and Arun, M (2023). A simple and efficient genetic transformation system for soybean (*Glycine max* (L.) Merrill) targeting apical meristem of modified half-seed explant. 3 Biotech 13, 293 https://doi.org/10.1007/s13205-023-03715-8 (Impact factor: 2.6)

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