Gibberellic acid-producing microorganisms and plant growth enhancement.

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***Abstract*- Gibberellic acid (GA_3), a crucial hormone for plants, regulates their growth and development. It is frequently utilized in horticulture, gardening, agriculture, and other fields. Other than plants, a number of bacteria and fungi strains can also create GA_3 as a secondary metabolite. Gibberellins are a wide family of plant growth hormones that were first identified in the 1930s. They are produced from geranylgeranyl diphosphate using the terpenes pathway and have an ent-gibberellin tetracyclic skeleton as its fundamental structural component. Only four of them exhibit biological activity, including gibberellic acid (GA_3), a natural plant growth regulator that is particularly effective for stem lengthening, seed germination, and enlarged fruit. It is derived from bacteria, fungi, and plants. High-purity broth samples are needed to measure gibberellic acid. Liquid chromatography is the most popular method for quantifying GA_3 from fermented broth, but micellar electrokinetic chromatography is also used. Gibberellic acid and GA_3 cannot be distinguished, resulting in inaccurate quantification. The finding that gibberellins cause the breakdown of the growth-inhibiting DELLA proteins supported the hypothesis that they work in plants by reducing growth restriction. The discovery of the gibberellin receptor from rice in 2005 shed light on the mechanism by which this is accomplished. The role of DELLA proteins as regulators of gene expression is a particular focus of current research on the activity of gibberellin.**

***Index Terms*— plants, crucial hormone, gibberellic acid, DELLA proteins, gibberellins, crops output, plant development, quantification, growth hormone**

I. INTRODUCTION

Bacteria are abundant in soil, interact with plant roots in the rhizosphere, and, in some cases, promote plant growth and development (Pandya & Desai, 2014). At various phases of plant development, low quantities of phytohormones influence a variety of crucial physiological responses in plants, including cell division, expansion, and differentiation, as well as organ senescence and abscission (Davies, 2004). Phytohormones are frequently found and function in trace amounts in plant tissues. Scientists have identified various forms of phytohormones, including auxins, cytokinins, abscisic acid, gibberellins, and ethylene, since the discovery of the first phytohormone, auxin, in 1926 (Rivier and Crozier, 1987). A natural plant growth hormone, gibberellic acid (GA_3) is a diterpenoid carboxylic acid that is a member of the gibberellins family. It is produced by plants and some microbes, including fungi and bacteria. Due to its characteristics related to plant development, GA_3 has interesting uses in the agro-industrial sector. In crops, orchards, and ornamental plants, GA_3 is used to promote fruit growth, stem elongation, flowering, the malting of barley, and other physiological effects that result from its interactions with other phytohormones. It also plays a role in seed germination, response to abiotic stress, fruit growth enhancement, stem elongation, and flowering (Camara et al., 2018). Currently, industrial-scale submerged fermentation of the fungus *Gibberella fujikuroi* is the main method used to create GA_3 (Santos et al., 2003). Additionally, it is produced by a variety of bacteria in culture medium, including *Azotobacter*, *Pseudomonas*, and *Azospirillium*, as well as from wild strains of fungi, including *Sphaceloma* sp., *Phaeosphaeria* sp., and *Neurospora* species (Rademacher, 1994).

II. History and structure of gibberellic acid:

The earliest reports on gibberellins were made by a team of Japanese researchers who concentrated some of their research on the *bakanae* disease, which was particularly bad for local farmers' rice (Hori, 1903). One or more *Fusarium* species are responsible for the *bakanae* disease. Numerous signs of this disease include stunting, root and crown rot, seedling blight, and the traditional etiolation and aberrant elongation caused by the fungal synthesis of gibberellins (Rademacher, 1994). Plant pathologists discovered that a chemical secreted by the pathogenic fungus *Gibberella fujikuroi* caused these symptoms in rice plants. By growing this fungus in a lab and analyzing the filtrate, Japanese scientists were able to extract impure crystals of two fungal "compounds" in the 1930s that had effects that encouraged plant development. One of these was given the name Gibberellin A since it was isolated from the fungus *Gibberella*. Scientists at Tokyo University extracted three distinct gibberellins from a sample of gibberellin A in the 1950s and termed them gibberellins A1, A2, and A3. This first nomenclature of gibberellins A1 (GA_1), GA_2, and GA_3 serves as the foundation for the numbering scheme for gibberellins utilized over the past 50 years (Gupta & Chakrabarty, 2013).

All biologically active GAs have a skeleton made up of 19 carbon atoms (C_{19}-GAs), although GAs can also have skeletons made up of 20 carbon atoms (C_{20}-GAs) (Tarkowská et al., 2014). There are now over 140 different GA molecules that have been identified from plants and microbes. However, only a small number of these molecules are biologically active, and the majority are only minor precursors. Those with the most commercially available biological properties are GA_3, GA_4, and GA_7 (MacMillan 2001; Kawaide 2006). These GAs contain three structural characteristics in common: i) a hydroxyl group on C-3β, ii) a carboxyl group on C-6; and iii) a lactone between C-4 and C-10. The C-2 and/or C-3 positions of the 3β-hydroxyl group are interchangeable with different functional groups. Examples of bioactive GAs without a hydroxyl group on C-3β are GA_5 and GA_6. The fact that GA_1 is present in

a variety of plant species shows that it is a typical bioactive GA (Ghosh & Halder, 2018). Chemically characterized, GA_3 ($C_{19}H_{22}O_6$, CAS 77-06-5, MM = 346.37) is a tetracyclic dihydroxy-γ-lactone acid with a C1-C2 double bond, C10 γ- lactone ring, and an OH group in C13. It is a white, crystalline powder with a melting point of between 233 and 235 °C, and it is sparingly soluble in petroleum ether, benzene, and chloroform. It is soluble in alcohol, acetone, ethyl acetate, and butyl acetate. Only 5 g L-1 of GA_3s may dissolve in water, which is a very low amount. It is stable in dry environments but easily breaks down in hot environments, environments with an alkaline pH, and in watery solutions. At 20 °C and 50 °C, it has a half-life in aqueous solutions of roughly 14 days and 2 days, respectively. GA_3 can be hydrolyzed to gibberellic acid in aqueous solutions through a purely chemical process; however, in the presence of microbes, GA_3 can also be metabolized during C-limitation (Camara et. al., 2018).

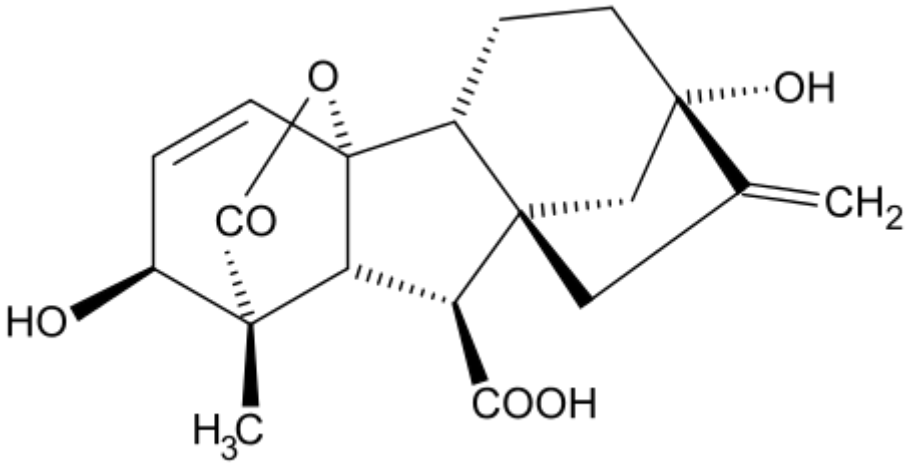

Figure 1: Structure of gibberellic acid

III. Bacteria that are known to increase crop output and plant development:

This tendency originated in wealthy nations, but the "green revolution" of the 1960s brought it to the "third world." However, a variety of biological systems have been severely harmed by the steadily rising usage of inorganic fertilizers. As a result, more recently, N2-fixing bacteria that are more "environmentally friendly" have come under increased scrutiny as a means of boosting agricultural productivity (Okon and Labandera-González 1994). A low concentration of GA_3 in foliar sprays has been studied, and the performance, quality, and salt tolerance of several fruits and seed vegetables in soil-based and hydroponic growth methods have shown good results (Miceli et. al., 2018). The enhanced 15N uptake seen following inoculation of wheat roots has been attributed to the synthesis of gibberellin by *Azospirillum* species and *Bacillus* species (Kucey, 1988). Inoculation with various *Azospirillum* strains enhanced levels of GA_3 in maize roots, but non-inoculated seedlings primarily contained conjugated GA_3. Application of GA_3 to the roots in quantities comparable to those produced by the microorganisms improved root growth in maize seedlings. In addition, inoculated with *Azospirillum* sp. Rice and maize seedlings revealed a reversal of dwarfism, both genetic and caused by inhibitors of gibberellin production, demonstrating the bacteria's endophytic presence (Bottini et. al., 2004). Gibberellins may be important players in a variety of metabolic processes that affect these traits, including the synthesis and breakdown of chlorophyll, assimilate translocation, nitrogen metabolism, and nitrogen redistribution (Miceli et. al., 2018). At germination, the synthesis of GA is dramatically increased in seeds, and its presence is necessary for germination to take place. Gas actively encourages cell elongation in adults and seedlings. Additionally, Gas supports the transition from vegetative to reproductive development and is necessary for pollen function during fertilization (Toungos, 2018). It is more crucial to produce blooms that are of high grade. Their exogenous use aids in enhancing several economically significant and marketable traits of flowering plants. The production of superior flowers is extremely important. Their exogenous application helps flowering plants develop a number of economically valuable and marketable features (Castro-Camba et. al., 2022). Both the *Bacillus pumilus* and the *Bacillus licheniformis* strains were discovered in the rhizosphere of *Alnus glutinosa* L. Gaertn., both exhibit potent growth-promoting properties. The application of extracts from media containing both bacteria and exogenous GA_3 significantly restored the dwarf phenotype that Paclobutrazol, an inhibitor of gibberellin production, caused in *A. glutinosa* seedlings. GC-MS analysis of these extracts revealed the presence of GA_1, GA_3, GA_4, and GA_{20}. Additionally, Inoculation with *Bacillus licheniformis* and *Bacillus pumilus* promoted the growth of Pinus pinea plants through the generation of bacterial gibberellin (Bottini et. al., 2004). Due to their direct effect on agricultural performance, Gas has enormous relevance. The analysis of dwarf wheat and rice variants revealed the role of GA in these plants' phenotypic outcomes (Castro-Camba et. al., 2022).

IV. Quantification:

1. Spectrometry:

By using hydrogen chloride to acidify the broth, GA_3 is changed into gibberellic acid. To avoid interferences during the measurement, this method does, however, need for high purity broth samples, particularly when using undefined fermentation materials (Camara et. al., 2018).

2. Chromatography:

The most popular chromatographic method for quantifying GA_3 from the fermented broth is high-performance liquid chromatography with UV detection. Liquid chromatography has been used to quantify GA_3 utilizing a variety of detectors, including

ultra-performance liquid chromatography followed by ESI-MS/MS, HPLC combined with fluorescence detection, and liquid chromatography-electrospray tandem with mass spectrometry. To determine GA_3 from fermentation broths, micellar electrokinetic chromatography was investigated as an alternative to HPLC (Camara et al., 2018).

3. Fluorescence method:

The interaction between sulfuric acid and the sample at 4 °C produces GA_3, which is quantified using fluorometric analysis. The issue with this method is that gibberellic acid and GA_3 cannot be distinguished, which could result in quantification errors (Camara et al., 2018).

V. Future Opportunity:

The US alone spent US$20.8 billion on decorative plants in 2006, underscoring the significant potential for growth and sales of both new and current ornamental plants. With the advancement of floriculture-based technologies, such as the petunia DNA microarray chip, it is now possible to find novel genes and, in the near future, to create plants with innovative, consumer-pleasing features. Key aspects of growth and development are governed by plant hormones; artificial growth regulators work by altering or replacing the actions of natural hormones. A genetic engineering strategy might lessen the need for artificial growth regulators. The significant costs of creating novel cultivars and acquiring intellectual property rights to shield new variations from competition and exploitation necessitate a sizeable financial investment. Tanaka and others suggested that the introduction of novel genetically modified features would help the floriculture sector. There is unquestionably great promise for controlling GA signaling in plants, and proof-of-concept experiments have already been carried out. Other growth hormones, such as auxins, cytokinins, ethylene, brassinosteroids, jasmonic acid, salicylic acid, and polyamines, also have an impact on plant development; therefore, it is possible that genetically modified ornamentals would target the biosynthesis pathways for these hormones. Indeed, altering many hormonal pathways may be necessary to change a plant's size. Future studies must focus on finding inducible promoters that are tissue- or species-specific and may, for instance, stimulate gene expression in vegetative tissues like stem internodes without interfering with blooming or seed formation (Bhattacharya et. al., 2010).

Understanding the regulation of GA metabolism, which predominantly happens in the stages catalyzed by ODDs, has been the focus of recent research. Although there are many molecular elements involved in GA metabolic control, our knowledge of these mechanisms is still far from complete. The mechanisms governing GA homeostasis in particular are proving to be remarkably complex, probably in part because of the promiscuous connections of the DELLA proteins that act as its mediators. A rapidly developing field of study is currently focused on understanding the nature of DELLA-mediated signaling and identifying the numerous partners of these proteins. There is growing evidence that suggests DELLAs may act as hubs for various hormone signaling pathways, creating a pathway by which these pathways may affect GA metabolism. However, it is evident that auxin signaling and, in certain cases, that of ABA directly alter GA biosynthesis without the help of DELLAs. Moreover, there is still a great deal to learn about the sites, particularly at the cellular level, where GA is created. Renewed interest in the GA movement is being sparked by mounting evidence that Gas can act remotely from its source. GA movement can take place by diffusion between nearby cells or over long distances within the vasculature, particularly the phloem. In order to investigate GA production and activity at a higher tissue resolution than is currently possible, more advanced physicochemical and molecular techniques are needed. Though most parts of plant development are influenced by GA signaling, the specificity of the expression domains and subsequent physiological function of the ODD paralogues in GA metabolism has made it possible to identify GA-deficient mutants with useful agricultural features. For example, the *sd1* Mutant of rice, lacking *OsGA20ox2*, and *le* pea, with a mutant Allele of *PsGA3ox1*, have reduced stem height, but normal fertility (Jahuddin et.al., 2019).

Understanding how GA signaling is controlled requires a thorough mapping of the locations of GA production and action in plants. Although liquid chromatography-mass spectrometry and, more recently, GC-MS have significantly increased the sensitivity of physicochemical approaches for analyzing GA concentrations, they are still insufficient to measure the amounts of Gas and precursors at the cellular level. The further characterization of GA transporters as well as the development of in situ methods for identifying the cells that create, accumulate, and respond to bioactive Gas are essential goals (Davies, 2004).

The Information presented here, taken together, lends credence to the idea that, in the effort to practice sustainable agriculture through environmentally friendly methods, the potential of microorganisms that produce Gas may be able to make a significant contribution by promoting the health of various crops (Rivier & Crozier, 1987).

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