

# **Recent Advancements In Gibberellic Acid Formulation Techniques.**

## **ABSTRACT**

Gibberellins comprises of a broader family of plant advancement hormones found during the 1930s, which are synthesized by implies of the terpenes course from the geranylgeranyl diphosphate and incorporate a crucial structure formed by an ent-gibberellane tetracyclic skeleton. Among them ( $GA_3$ ), which acts as a characteristic plant development controller, especially for stem extending, seed germination, and extended common item degree. It can be gotten from plants, organisms, and microbes. There are as well a couple of reports roughly microalgae  $GA_3$  makers. Life forms, especially *Gibberella fujikuroi*, are favored for  $GA_3$  generation through submerged maturing or solid-state development. Various factors may impact its generation, a few of which are related to the control and scale-up of maturing parameters. Particular  $GA_3$  items are open on the advertise. They can be found in liquid or solid dosage forms containing because it were  $GA_3$  or a mix of other normal dynamic gibberellins, which can be employed in agriculture, horticulture & floriculture crops.

*Keywords: Plant advancement hormones, Gibberella fujikuroi, tetracyclic skeleton*

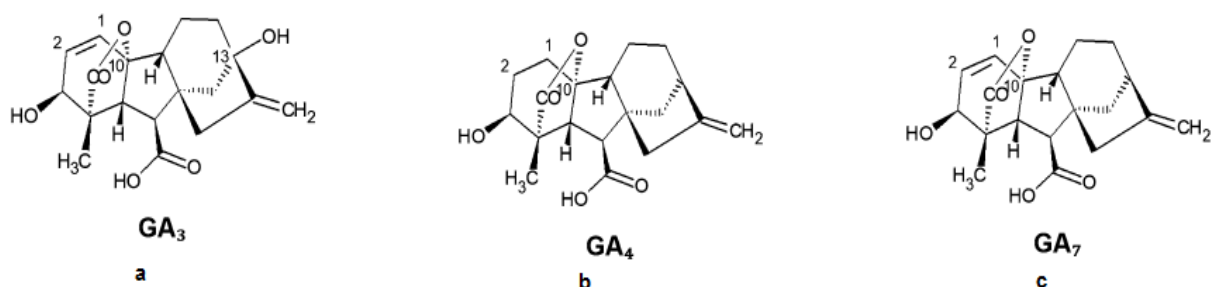
## **1. INTRODUCTION**

$GA_3$  is connected to crops, plantations, and decorative plants, where it plays a part in seed germination reaction to abiotic stress, natural product development enhancement, stem stretching[1], blossoming [2], the malting of grain[3], and other physiological impacts that happen in its interaction with other phytohormones[4-5]. Gibberellic acid ( $GA_3$ ) may be a diterpenoid carboxylic acid that belongs to the gibberellins family and acts as a normal plant growth hormone. Plants and a few microorganisms, such as fungi and microscopic organisms, deliver it.  $GA_3$  has promising applications in the agro-industrial segment due to its properties related to plant development. Since it was found, studies have been centered on upgrading its synthetic processes, boosting its efficiency, and diminishing its cost, which sometimes restricts the utilization of this imperative development hormone.

This survey centers on general perspectives of  $GA_3$  production through biotechnological forms, the talk approximately the main recuperation options and detailing forms, its market accessibility, and the natural impacts in plants.

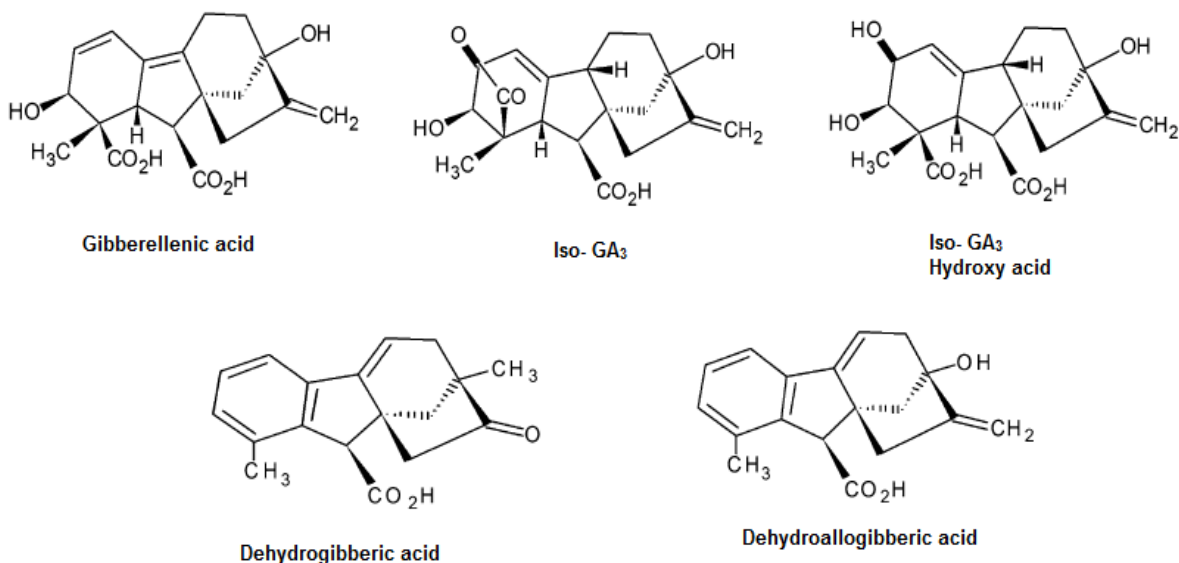
## 1.1 General chemistry

Gibberellins (GAs) were found in Japan during 1930s when a bunch of researchers and agriculturists started to ponder a disease influencing rice areas. This malady was characterized by intemperate stem development, yellowing of the influenced areas, and a low seed production was observed [6].  $GA_3$  is a white crystalline powder with a melting point of around 233–235 °C that is soluble in liquor, acetone, ethyl acetate, and butyl acetate, and it is sparingly soluble in petroleum ether, benzene, and chloroform.  $GA_3$ 's solubility in water is low, reaching only 5 g L<sup>-1</sup>. It readily decomposes at higher temperatures, at alkaline pH and in aqueous solutions with a half life of approximately 14 days at 20 °C & 2 days at 50 °C [7-8]. The lack of stability can be associated with a C1–C2 double bond in its chemical structure, making the molecule more reactive [9] (Fig.1a).  $GA_7$  also possesses the double bond at C1-C2 and has same lacks the stability due to the presence of double bond. The most stable of the three GAs is  $GA_4$  (Fig. 1b). The removal of the  $\gamma$ -lactone ring has also been linked to the biological inactivation of  $GA_3$  [10] and as a result, the existence of the  $\gamma$ -lactone ring and the C1–C2 double bond in the  $GA_3$  structure is essential for its biological activity.  $GA_3$  hydrolysis to gibberellic acid can occur in aqueous solutions in a solely chemical process; however,  $GA_3$  can be metabolised during C-limitation[11] in the presence of microbes. Different  $GA_3$  breakdown products can be generated depending on the temperature, reaction duration, and pH of the solution, resulting from changes in the molecule's structure, and its "gibberellin-like" biological activity can be diminished or completely lost the metabolic products are as shown in (fig.2)



**Fig.1. Chemical structures of bioactive gibberellins  $GA_3$  (a) Gibberellic acid,  $GA_4$  (b) &  $GA_7$  (c)**

$GA_3$  (C<sub>19</sub>H<sub>22</sub>O<sub>6</sub>, CAS 77-06-5, MM = 346.37) is a tetracyclic dihydroxy-lactone acid that has a C1–C2 double bond, a C10  $\gamma$ -lactone ring, and also a OH group in C13 (Fig. 1a).



**Fig.2. Metabolic products of GA in aqueous solutions under different conditions of pH & temperature**

## 1.2 Biosynthesis

Plants and fungi use the terpenes pathway to synthesise GA from geranylgeranyl diphosphate. This path has been thoroughly discussed by several researchers. As a result, GA synthesis will be explored briefly in this review. The stages of GA biosynthesis are as described hereunder.[12-14]

### Stage 1: geranylgeranyl diphosphate conversion to *ent*-kaurene

Geranylgeranyl diphosphate is made up of four isoprenoid molecules bonded together to produce a 20-carbon linear molecule (GGPP). This molecule is converted to entcopalyl-diphosphate (CPS) by an ent-copalyl diphosphate synthase (CPS), which is then converted into the tetracyclic compound *ent*-kaurene by an *ent*-kaurene synthase (KS).

### Stage 2: *ent*-kaurene conversion to GA<sub>12</sub>

Plants' *ent*-kaurene oxidase (KO) and fungi's P450-4 catalyse the successive oxidation of *ent*-kaurene in C-19 to form *ent*-kaurenoic acid, which is then converted to GA<sub>12</sub>- aldehyde by an *ent*kaurenoic acid oxidase (KAO) in plants and P450-1 in fungi.

### Stage 3: GA<sub>12</sub> conversion to other GAs

GA<sub>12</sub>- aldehyde is first transformed to GA<sub>12</sub> in plants, and subsequently to GA<sub>9</sub> by the action of GA<sub>20</sub>-oxidase, which is responsible for the formation of C19- GAs. In a separate process, GA<sub>12</sub> is 13 hydroxylated to produce GA<sub>53</sub>, which is then transformed to GA<sub>20</sub> by the action of C20-oxidase. Then, by adding a 3-beta hydroxyl group to GA<sub>20</sub> and GA<sub>9</sub>, GA<sub>3</sub>-oxidase transforms them into GA<sub>1</sub> and GA<sub>4</sub>, respectively. GA<sub>3</sub> is generated when GA<sub>20</sub> is converted to GA<sub>5</sub> by the enzyme GA<sub>3</sub>- oxidase. This stage varies by species and is influenced by environmental factors.

acid is a

CC(C)C

**Fig.3. The biosynthesis process for gibberellin (GA) in fungi and plants begins with GGDP (geranylgeranyl diphosphate). In fungus and plants, the first GA created is GA<sub>14</sub> and GA<sub>12</sub>. In fungi, CPS (ent-copalyl diphosphate synthase), KS (ent-kaurene synthase), and P450 (cytochrome P450 oxidoreductase) are involved, while in plants, CPS, KS, KO (ent-kaurenoic acid oxidase), KAO (ent-kaurenoic acid oxidase), and GA oxidase (GA13ox)**

## 2. FORMULATION ASPECTS OF GIBBERELIC ACID

GA<sub>3</sub> is found in a variety of commercial formulations, either alone or in combination with GA<sub>4</sub> and GA<sub>7</sub>. They come in a variety of forms, including liquid, soluble powder, wettable powder, tablet, and water-dispersible granular. The table no.1 lists some of the product names that include GA<sub>3</sub> as well as their producers.

The relative lack of stability of GA<sub>3</sub> in the presence of water is connected with liquid formulations, which may result in a short shelf life. Some solvents are used in this way to prevent GAs from degrading, but this could result in combustible formulations that would necessitate extreme caution when packaging, transporting, and storing them. Solid formulations appear to be a safer technique due to the disadvantages of liquid formulations, but they also have some drawbacks, such as dust while pouring, moving, or measuring; the likelihood of creating residues in the tank and plants; and the chance of lump formation.[15]

### 2.1. Liquid formulations

GA<sub>3</sub> is dissolved in an alcohol solution, such as isopropanol, methanol, or ethanol, at a concentration of around 4% (w/v) for most commercial products. Some additives and/or adjuvants are also used to boost product stability, target a molecule's protective action, extend shelf life, or increase nutritional components in plants.[16-17]

**Table1. Different types of GA<sub>3</sub> products available in the international market and their manufacturers**

Producers	Trademarks	Formulation type
Orion Crop Protection	PastureGibb®	Liquid
NuFarm Americas	GibGro® 4LS	
Valent BioSciences Corporation	Progibb® 4%/Rizup® 4SL/Release LC	
NuFarm Americas	GibGro®	Soluble powder/ wettable powder
Fertco	Gibb-gro®	
Valent BioSciences Corporation	Progibb® 2x/Release®/Berelex® 2x	
Valent BioSciences Corporation	Progibb® tablet/Berelex® tablet	Tablet
Ravensdown	Express®	Soluble granule
Valent BioSciences Corporation	Progibb® SG/Progibb® 40 SG/Activol® 40SG/Berelex® 40SG/Ryzup® 40SG/Ryzup® SmartGrass 40WSG	
Sum Farm New Zealand Limited	Gibb-star®	

The influence of adjuvants on the stability of cleared GA<sub>3</sub> extract was investigated. Adjuvants extend the shelf life of products by preventing or delaying ingredient degradation and promoting extra properties in the final product, such as high stability. After screening tests revealed that the liquid formulation was defined with ethanol (50 percent v/v) and Tween 20 (2.5 percent), which promoted a better conservation of GA<sub>3</sub> activity in accelerated stability tests, the liquid formulation was defined with ethanol (50 percent v/v) and Tween 20 (2.5 percent). The hormone formulation was subsequently subjected to a temperature of 50°C for 14 days, with GA<sub>3</sub> activity remaining at roughly 67 percent. The rapid decline in GA<sub>3</sub> activity was most likely caused by its degradation, which was accelerated by the high temperatures utilised in the

experiment. When exposed to harsh conditions, such as high temperatures and pH, the GA<sub>3</sub> molecule decomposes quickly.

Other chemicals such as gibberellic acid, iso-gibberellic acid, and dehydroallo-gibberic acid may occur under these conditions. GA<sub>3</sub> isomerization can also occur in weak alkaline conditions. This plant growth hormone has little or no biological function in these forms. In fact, the active biomolecule was put under extreme stress during the accelerated storage test. The goal of this test was to use heat to imitate typical long-term ageing of a formulation. In this test, a loss of up to 5% of the active component ensures a shelf life of at least two years.[18]. A long-term stability test or alternative circumstances should be utilised to evaluate the product's shelf life in the event of very sensitive active components [19]. As a result, the long-term stability of the GA<sub>3</sub> formulation was tested, and it was found to retain 100% of its activity after 6 months at room temperature. The unformulated extract, on the other hand, lost more than half of its activity and revealed some contamination, which could have contributed to the activity reduction because microbes can metabolise the biomolecule in the absence of carbon sources.[20]. These findings are consistent with previous studies that show low GA<sub>3</sub> stability in aqueous solutions.

## **2.2. Powder formulations**

Some formulations use solvents with high levels of volatile organic compounds that are harmful to the environment in order to overcome solubility difficulties. Isopropyl alcohol and methyl alcohol, for example, have serious drawbacks such as flammability and toxicity, which limit their use in production, packaging, labelling, shipping, and warehousing. The eye and skin are both corroded by tetrahydrofurfuryl alcohol (THFA). Developing soluble powder formulations using GA<sub>3</sub>, GA<sub>4</sub>, and GA<sub>7</sub> is one technique to tackle the solubility concerns. When mixed with water, these powder formulations dissolve quickly and form genuine solutions. No more mixing or agitation of the tank-mix is required once the solution has formed.

Making a wettable powder is another technique to get around the solubility concerns. A wettable powder formulation is one that is dry and finely milled. The active component is coupled with a finely ground dry carrier, commonly a mineral clay, as well as other additives that improve the powder's capacity to be suspended in water in this sort of formulation. When the wettable powder is mixed with water, a suspension is created, which is then sprayed on. To avoid the settling of insoluble compositions, the spray liquid must frequently be continually mixed. However, when handling wettable powders and soluble powder formulations, such as pouring, transporting, or measuring them, they tend to produce dust. This dust could be hazardous to human health. Furthermore, powder formulations have a weak wetting ability and solubilize slowly when mixed with water. In a tank-mix, powder formulations take longer to wet, disperse, and solubilize. Due to the formation of lumps or partially solubilized spray solutions, the plant growth regulator will be distributed unevenly in the tank-mix, potentially resulting in lower field performance. Spray tank adjuvants can sometimes generate foam in the spray tank, which can impact the wetting and solubility of wettable and soluble powders. Wettable powder formulations will also leave insoluble residues in the tank, as well as on sprayed foliage and fruit. [21]

## **2.2. Tablet formulations**

A tablet is another sort of agricultural preparation. Tablet formulations are dose delivery systems that have been pre-measured. They're great for small spaces or for ornamental purpose. Effervescent tablet formulations dissolve in water over a two to ten minute period, depending on the kind and size of the tablet. Tablets, on the other hand, typically contain only 0.1 to 1 gram of active substance per pill. They aren't well suited to large-scale field activities. Furthermore, effervescent tablets are sensitive to dampness, can be slow to dissolve, and are costly.[22]

### 2.3. Water dispersible granules

A water-dispersible granule is another type of agricultural preparation. Wettable granules or dry flowables are other names for water-dispersible granules. This formulation is comparable to a wettable powder, but the active ingredient is in the form of a dispersible granule. The water-dispersible granules are dispersed in water and agitated to form a suspension, ready for spray application. Agricultural chemicals are available in a variety of water-dispersible granular forms [23.] When water-dispersible granules are introduced to aqueous solutions, they generate suspensions. To thoroughly disperse the resultant Suspension, it must be stirred for a length of time. During application, agitation or by-pass recirculation of the tank mix must be maintained. Some common excipients which are employed for the gibberellic acid formulations are enumerated in table no.2.

**Table.2. Excipients employed in solid & liquid GA formulations**

Category	Excipients
Antioxidants	Gallate (propyl, octyl and dodecyl), ascorbic acid, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), t-butylhydroquinone (TBHQ)
Antifoam	Silicone emulsions full of silica
Encapsulating agents	Gums (arabic, alginate and carrageenan), proteins (whey and milk proteins, gelatin), carbohydrate (maltodextrin, starch, sucrose, glucose, trehalose, pectin)
Preservatives	Sulfur dioxide, benzoic acid, sorbic acid, propionic acid, sodium and potassium salts, nitrite and nitrate of sodium and potassium
Surfactants	Nonionic: ethoxylated esters of sorbitan, fatty acid esters, glucose and sucrose esters, ethoxylated alcohols, ethoxylated alkylphenols, ethoxylated fatty acids Anionic: phosphate esters, sulfate and sulfonated oils, sulfates and sulfonates ethoxylated alkylphenols
Diluents (Liquid formulations)	Polyethylene glycol, ethanol, isopropyl alcohol, methyl alcohol, glycerol, propanol, <i>n</i> -butanol, <i>n</i> -amyl alcohol, acetone, methyl butyl ketone, ethyl lactate, <i>n</i> -butyl lactate, and propylene glycol
Diluents (solid formulation)	Sorbitol, mannitol, lactose, dextrose, starch, limestone, sucrose, maltose, maltodextrin, and fructose
UV protectant	Benzophenone-3 and ethylhexyl methoxycinnamate
Anti-caking	Silica, talc, and limestone
Binder	Maltodextrin, lecithin and polyvinylpyrrolidone

### 2.3. GA<sub>3</sub> beads

The study of GA<sub>3</sub> beads with ALG and ALG/KEF systems was carried out as a new approach for future plant growth hormone application and controlled release in the fields. This is the first time kefirin has been used as a polymeric matrix for GA<sub>3</sub> encapsulation has been reported. The GA<sub>3</sub> molecule must be protected in order to maintain its biological activity. Encapsulation has been reported as an alternative for protecting plant hormones from environmental conditions, which increases efficiency and promotes controlled release. A promising combination of ALG and KEF for the production of GA<sub>3</sub> beads was proposed in this preliminary study.

GA<sub>3</sub> encapsulation efficiency was approximately 70% for ALG beads and 60% for ALG/KEF beads, respectively. Another molecule, Celecoxib [24], was encapsulated in ALG beads with an encapsulation efficiency of about 40%. Another study used ALG/KEF beads to encapsulate ciprofloxacin [25] and reported an encapsulation efficiency of 80%. Variations in encapsulation efficiency between different

matrices of beads could be attributed to chemical interactions between each polymer and GA<sub>3</sub>. When compared to ALG beads, ALG/KEF beads had a lower encapsulation efficiency. Kefiran may be to blame for the decrease in polymer interactions with GA<sub>3</sub> because it competes with GA<sub>3</sub> for alginate binding. As a result, an inefficient matrix was created in this case.

In aqueous solution, ALG and ALG/KEF beads had a spherical shape with a diameter of about 4 mm and 3.5 mm for the ALG system and ALG/KEF beads, respectively, with or without GA<sub>3</sub>. SEM was used to examine GA<sub>3</sub> beads. The shrinkable matrix observed and the loss of the beads' spherical morphology are most likely related to the fact that the beads were freeze-dried prior to SEM analysis. When compared to ALG beads, the inclusion of KEF in the bead matrix promoted a smoother surface. Some have reported that KEF could help to stabilise the gel matrix structure. KEF and ALG beads had a porous surface with only a few cracks. There have been no reports of GA<sub>3</sub> encapsulation in ALG or ALG/KEF beads up to this point. Kefiran is a promising biopolymer for use as a polymeric matrix due to antibacterial and antifungal properties that could prevent contamination and increase product shelf-life. [26]

Because of the carboxylic groups from uronic acids contained in its linear structure, alginate is a biopolymer with a negative charge. When the biopolymer is introduced to a CaCl<sub>2</sub> solution, it undergoes a polymerization process. The polymeric matrix is formed when the carboxylic acid groups combine with the Ca<sup>2+</sup> ions, generating a reversible gel known as a "egg-box" structure. GA<sub>3</sub> has carboxylic acid groups in its chemical structure, so it can be incorporated into the polymeric mesh created by ALG and Ca<sup>2+</sup> ions through the same chemical interaction. There are a few factors to consider when considering future GA<sub>3</sub> controlled releases in field applications [27]. Although ALG beads are stable at low pH, they may swell in basic solutions, causing destabilisation and erosion. Furthermore, when monovalent cations and complex anions such as phosphate and citrate are present in the media, calcium alginate gels become unstable. As a result, certain conditions of the medium in which the hormone will be released are critical for ensuring the stability of the beads and a good release of the biomolecule [28]. Changes in the medium's pH are linked to the release of molecules from alginate beads. This is explained by the effect of pH on the charge of the molecules, which affects the hormone-polymer interactions. The electrostatic interactions between the biopolymers and the hormone could define the release behaviour. As a result, fresh experiments to assess the stability of beads and the release behaviour of GA<sub>3</sub> are currently underway in order to explicate these crucial details for future use.

### **3. ECONOMIC ASPECTS OF GIBBERELIC ACID**

The growing worldwide population's desire for food has inspired study into how to increase the amount and quality of agricultural produce. GA<sub>3</sub> serves a significant economic role as a plant growth regulator because of its wide variety of applications, from crops to fruits, which improves production. GA<sub>3</sub> output is estimated to be roughly 100 tonnes per year, with a market worth of US \$ 100 million, with around three-quarters used for plant production and the other quarter for malting [29]. GA<sub>3</sub> is used in viticulture all over the world to increase fruit size. However, its use is not restricted to viticulture; some producers have identified more than 40 different crops for which GA preparations can be used to improve quality and value, including fruits, vegetables, and cereals. GA<sub>3</sub> is one of the most popular and widely used plant growth regulators (PGR). Its widespread use has the benefit of increasing crop productivity, quality, and value. Due to the high expenses of development and manufacture, only a few companies are still working on PGRs, restricting their widespread application. Herbicides, insecticides, and fungicides continue to be the focus of these companies' investments. However, as a result of the drive for a more sustainable agriculture, the production and usage of GA<sub>3</sub> products is predicted to grow.



#### 4. FUTURE PROSPECTUS

The definition of stable liquid or solid formulations is one of the key obstacles to its deployment. Different GA<sub>3</sub> commercial products have been documented and are accessible on the global market, allowing it to be used in a wide range of cultivars. The quest for novel and low-cost GA<sub>3</sub> production techniques will undoubtedly expand its applicability, benefiting the quality and productivity of various cultivars all over the world, particularly in India, which is one of the world's most important agriculture-based economies.

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