



Current advances in gibberellic acid (GA₃) production, patented technologies and potential applications

Marcela C. Camara¹ · Luciana P. S. Vandenberghe¹ · Cristine Rodrigues¹ · Juliana de Oliveira¹ · Craig Faulds² · Emmanuel Bertrand² · Carlos R. Soccol¹

Received: 31 January 2018 / Accepted: 25 July 2018 / Published online: 1 August 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Main conclusion Gibberellic acid is a plant growth hormone that promotes cell expansion and division. Studies have aimed at optimizing and reducing production costs, which could make its application economically viable for different cultivars.

Gibberellins consist of a large family of plant growth hormones discovered in the 1930s, which are synthesized via the terpenes route from the geranylgeranyl diphosphate and feature a basic structure formed by an *ent*-gibberellane tetracyclic skeleton. Among them, only four have biological activity, including gibberellic acid (GA₃), which acts as a natural plant growth regulator, especially for stem elongation, seed germination, and increased fruit size. It can be obtained from plants, fungi, and bacteria. There are also some reports about microalgae GA₃ producers. Fungi, especially *Gibberella fujikuroi*, are preferred for GA₃ production via submerged fermentation or solid-state fermentation. Many factors may affect its production, some of which are related to the control and scale-up of fermentation parameters. Different GA₃ products are available on the market. They can be found in liquid or solid formulations containing only GA₃ or a mixture of other biological active gibberellins, which can be applied on a wide variety of cultivars, including crops and fruits. However, the product's cost still limits its large and continuous application. New low-cost and efficient GA₃ production alternatives are surely welcome. This review deals with the latest scientific and technological advances on production, recovery, formulation, and applications of this important plant growth hormone.

Keywords Plant growth regulators · *Fusarium fujikuroi* · Submerged fermentation · Alternative substrate · Downstream · Formulation

Introduction

Gibberellic acid (GA₃) is a diterpenoid carboxylic acid that belongs to the gibberellins family and acts as a natural plant growth hormone. Plants and some microorganisms, such as fungi and bacteria, produce it. GA₃ has promising applications in the agro-industrial sector due to its properties

related to plant development. Since it was discovered, studies have been focused on enhancing its process yield, boosting its productivity, and reducing its cost, which sometimes restricts the use of this important growth hormone.

GA₃ is applied to crops, orchards, and ornamental plants, where it plays a role in seed germination (Finch-Savage and Leubner-Metzger 2006; Chen et al. 2008; Urbanova and Leubner-Metzger 2016), response to abiotic stress (Colebrook et al. 2014), fruit growth enhancement (Li et al. 2011), stem elongation (Dayan et al. 2012; Wang et al. 2017), flowering (Sharma and Singh 2009; Muñoz-Fambuena et al. 2012), the malting of barley (Briggs 1963), and other physiological effects that occur in its interaction with other phytohormones (Steffens et al. 2006; Hedden and Sponsel 2015). This high-value molecule is mainly produced via submerged fermentation (SmF) through the secondary metabolism of

✉ Luciana P. S. Vandenberghe
lvandenberghe@ufpr.br

¹ Bioprocess Engineering and Biotechnology Department, Federal University of Paraná, Centro Politécnico, CP 19011, Curitiba, PR 81531-908, Brazil

² Aix-Marseille Université, POLYTECH Marseille, UMR 1163 Biotechnologie des Champignons Filamenteux, 163 Avenue de Luminy, 13288 Marseille Cedex 09, France

the fungus *Fusarium fujikuroi*. However, its industrial recovery and formulation are still little reported.

This review focuses on general aspects of GA₃ production through biotechnological processes, the discussion about the main recovery alternatives and formulation processes, its market availability, and the biological effects in plants.

General aspects

Gibberellins (GAs) were discovered in Japan in the 1930s when a group of scientists and farmers began to study a disease affecting rice fields. This disease was characterized by excessive stem growth, yellowing of the affected parties, and a lack of seed production (Taiz and Zeiger 2009). GAs can possess a skeleton with 20 carbon atoms (C₂₀-GAs) or 19 carbon atoms (C₁₉-GAs), in which all biologically active GAs possess 19 carbon atoms (Tarkowská et al. 2014). Currently, around 140 known different GA molecules have been isolated from plants and microorganisms, but only a part of them are biologically active, whereas most of them are precursors of minor importance (MacMillan 2001; Kawaide 2006). Those with the highest biological properties and that are commercially available are GA₃, GA₄, and GA₇.

GA₃ (C₁₉H₂₂O₆, CAS 77-06-5, MM=346.37) is chemically characterized as a tetracyclic dihydroxy- γ -lactone acid containing C1–C2 double bond, C10 γ -lactone ring and a OH group in C13 (Fig. 1a). It is a white crystalline powder with a melting point of around 233–235 °C that is soluble in alcohol, acetone, ethyl acetate, and butyl acetate, and it is sparingly soluble in petroleum ether, benzene, and chloroform. GA₃'s ability to dissolve in water is low, reaching only 5 g L⁻¹. It is stable in dry conditions and readily decomposes at high temperatures, at alkaline pH values, and in aqueous solutions. Its half-life in aqueous solutions is approximately 14 days at 20 °C and 2 days at 50 °C (Bruckner et al. 1991; Rodrigues et al. 2012a). Therefore, the lack of stability can be associated with a C1–C2 double bond in its chemical structure, making the molecule more reactive. According to Albermann et al. (2013), the same occurs with GA₇ (Fig. 1c), whereas GA₄ (Fig. 1b) is the most stable of the three GAs. It has also been suggested that the loss of the γ -lactone ring can lead to GA₃ biological inactivation (Pérez et al. 1996), and therefore, the presence of γ -lactone ring and C1–C2 double bond in GA₃ structure are crucial for maintaining its biological activity.

In aqueous solutions, GA₃ hydrolysis to gibberellic acid may occur in a strictly chemical process; however, in the presence of microorganisms, GA₃ can be metabolized during C-limitation (Hollmann et al. 1995). Depending on the temperature, the time of the reaction, and the pH of the solution, different GA₃ decomposition products can be obtained caused by changes in the structure of the molecule, and its “gibberellin-like” biological activity can be reduced

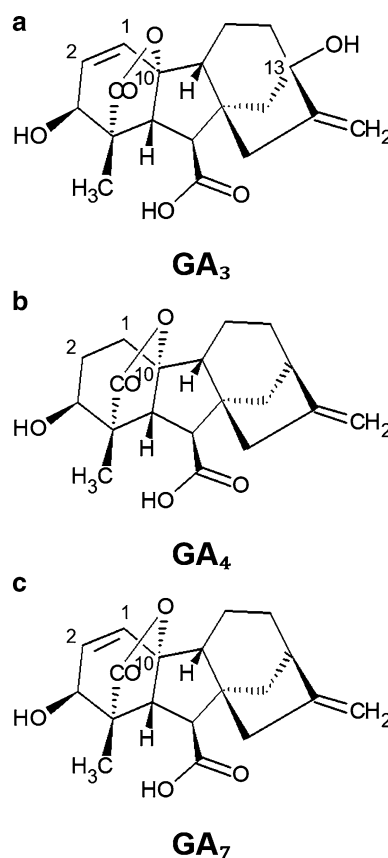


Fig. 1 Chemical structures of the bioactive gibberellins GA₃ (gibberellic acid) (a), GA₄ (b) and GA₇ (c)

or completely lost (Pérez et al. 1996). These compounds are shown in Fig. 2.

Biosynthesis

GA synthesis occurs via the terpenes route from geranylgeranyl diphosphate by plants and by fungi. Different researchers have extensively discussed this route. For this reason, GA synthesis will be shortly discussed in this review. More information can be seen in the work of Hedden and Phillips (2000); Tudzynski (2005); Yamaguchi (2008); Bömke and Tudzynski (2009); Hedden and Sponsel (2015). The stages of GA synthesis are described below.

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

Four isoprenoid molecules are linked together to form a linear molecule of 20 carbon atoms, known as geranylgeranyl diphosphate (GGPP). This molecule is transformed into *ent*-copalyl-diphosphate through the action of an *ent*-copalyl diphosphate synthase (CPS), which in turn is converted to

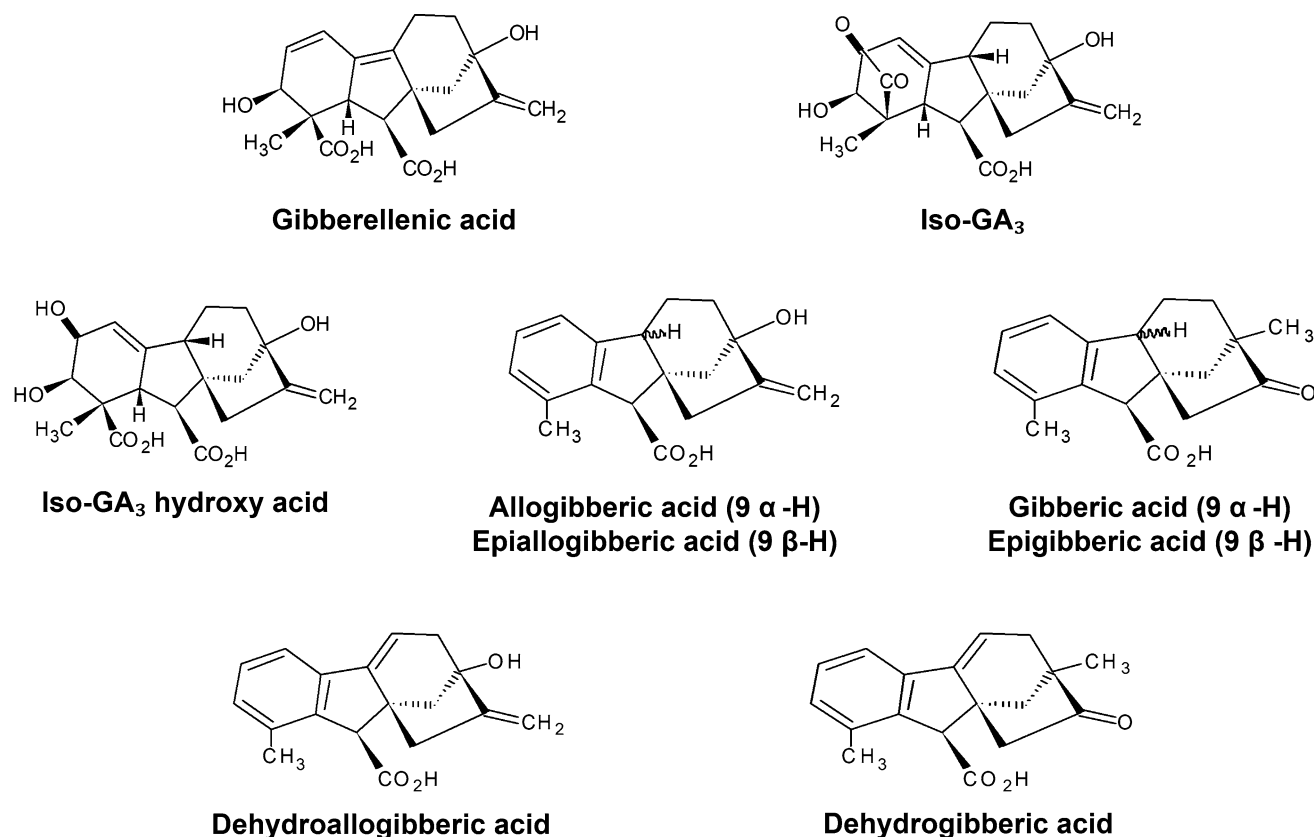


Fig. 2 Compounds formed from GA₃ decomposition in aqueous solution under different conditions of pH, temperature, or reaction time

a tetracyclic compound known as *ent*-kaurene through the action of an *ent*-kaurene synthase (KS).

Stage 2: *ent*-kaurene conversion to GA₁₂

The *ent*-kaurene oxidase (KO) in plants and the P450-4 in fungi catalyze the sequential oxidation in C-19 of *ent*-kaurene to produce *ent*-kaurenoic acid, which is subsequently converted to GA₁₂-aldehyde through the action of an *ent*-kaurenoic acid oxidase (KAO) in plants, and P450-1 in fungi.

Stage 3: GA₁₂ conversion to other GAs

In plants, GA₁₂-aldehyde is initially converted to GA₁₂ and then, through the action of GA₂₀-oxidase, which is responsible for the production of C₁₉-GAs, it is converted to GA₉. In a parallel pathway, GA₁₂ is also 13-hydroxylated for the production of GA₅₃, which is converted to GA₂₀ through the action of C₂₀-oxidase. Then, GA₃-oxidase converts GA₂₀ and GA₉, by the addition of a 3β-hydroxyl group, into GA₁ and GA₄, respectively. GA₃ is synthesized through the conversion of GA₂₀ into GA₅, by GA₃-oxidase. This stage differs between species and depends on environmental conditions.

In fungi, GA₁₂-aldehyde is 3β-hydroxylated to GA₁₄-aldehyde, which is oxidized to form GA₁₄. This last one, in turn, is converted to GA₄ through the oxidation of C20. The GA₄ is the first bioactive molecule that is formed and is desaturated to form GA₇, which is then converted to GA₃, by 13-hydroxylation. GA₁ is formed by the 13-hydroxylation of GA₄.

The biosynthesis pathways in plants and fungi during conversion of geranylgeranyl diphosphate to *ent*-kaurene, and subsequent conversion to GA₁₂-aldehyde, are similar. The pathways differ from the stage in which GA₁₂-aldehyde is converted to other GAs due to the order in which the steps of 3β-hydroxylation and 13-hydroxylation occur in plants and fungi. Plant and fungal GAs biosynthetic pathway is described in Fig. 3.

GA₃ production

Since 1935, the low amounts of GA₃ obtained from plants stimulated the production of this hormone through fermentation. Since then, efforts have been made to optimize its production, reduce costs, increase productivity, and discover new ways of production. In this way, solid-state fermentation (SSF) and SmF usually stand out in GA₃ production. SSF

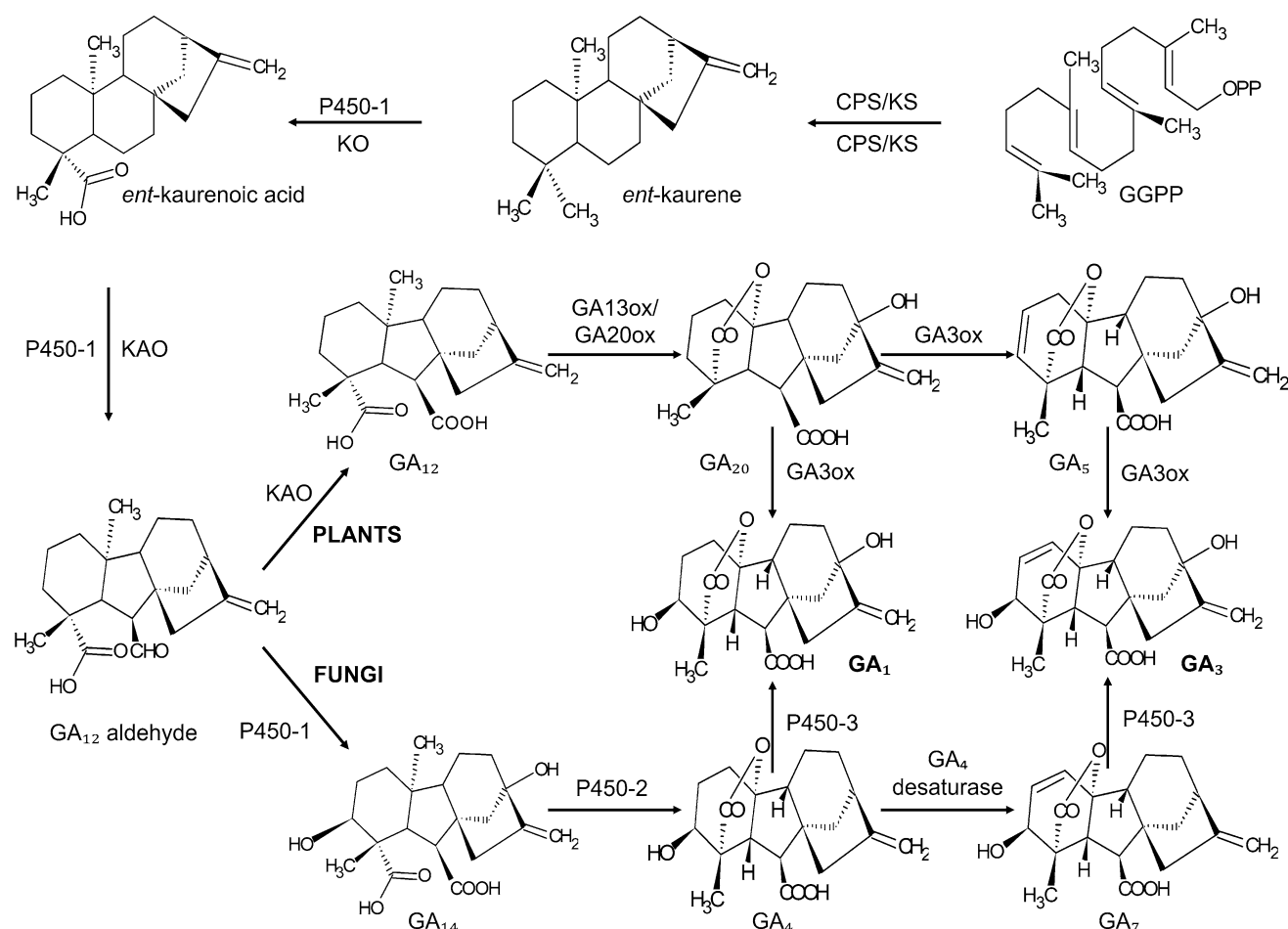


Fig. 3 Gibberellin's (GA) biosynthetic pathway in fungi and plants. The biosynthesis starts from GGDP (geranylgeranyl diphosphate) in both, fungi and plants. The route differs from GA₁₂ aldehyde, where the first GA formed is GA₁₄ and GA₁₂ in fungi and plants, respectively. The enzymes involved are CPS (*ent*-copalyl diphosphate syn-

thase), KS (*ent*-kaurene synthase) and P450 (cytochrome P450 oxidoreductase) in fungi, and CPS, KS, KO (*ent*-kaurene oxidase), KAO (*ent*-kaurenoic acid oxidase) and GA oxidase (GA₁₃ox, GA₂₀ox and GA₃ox) in plants

is a process in which the microorganism grows on a solid matrix in the absence of free water that serves as a support/substrate for microorganism development. Water is present only in a small amount that is sufficient for microorganism growth (Singhania et al. 2009).

SSF shows as advantages high product yields, low bacterial contamination, less wastewater generation, easier product recovery, and low-cost agro-industrial sub-products that can be used as substrates (Pandey et al. 2000; Selvaraj and Murty 2017). However, SSF scale-up is difficult due to problems with the control of fermentation parameters. Some alternative substrates were studied for GA₃ production through SSF, such as coffee husks (Machado et al. 2002), citric pulp (Rodrigues et al. 2016; de Oliveira et al. 2017), and others (Satpute et al. 2010). They are presented in Table 1.

SmF is the conventional and preferred technique, though it requires higher amounts of water, energy, and space, as

well as higher costs of product recovery. The microorganism is cultivated in a liquid medium, usually a synthetic medium. Alternative carbon sources such as rice flour (Escamilla et al. 2000; Uthandi et al. 2010), corn oil (Rios-iribe et al. 2016), and citric pulp (de Oliveira et al. 2017) have been used to compose the SmF medium for GA₃ production (Table 1).

Semi-solid-state fermentation (SSSF) is an unusual technique and little studied for GA₃ production. This technique overcomes some drawbacks presented by SSF and SmF, especially related to heat and mass transfer, nutrient availability, and production cost. In this case, the microorganism is cultivated in a liquid medium with solids in suspension (Selvaraj and Murty 2017). Agro-industrial sub-products can be used as substrate, promoting a support for fungi development and cost reduction. SSSF has been studied in the production of organic acids (Mai et al. 2016), enzymes (Gonzalez et al. 2013; Selvaraj and Murty 2017), and single cell oil (Economou et al. 2010).

Table 1 Production of GA₃ using different culture medium and fermentation strategies

Microorganism	Fermentation	Substrate	Production	Time	References
<i>Fusarium moniliforme</i>	SmF with immobilized cells	Glucose	680 mg L ⁻¹	12 days	Kahlon and Malhotra (1986)
<i>Fusarium fujikuroi</i>	SSF	Wheat bran	1.2 g kg ⁻¹	7 days	Kumar and Lonsane (1990)
<i>Fusarium fujikuroi</i>	SSF	Wheat bran	6.8 g kg ⁻¹	8 days	Agosin et al. (1997)
<i>Fusarium fujikuroi</i>	SSF- Fed-batch operation	Wheat bran	3 g kg ⁻¹	10 days	Bandelier et al. (1997)
<i>Fusarium fujikuroi</i>	SmF	Glucose and lactose	25 mg L ⁻¹	5 days	Tomasini and Fajardo (1997)
	SSF	Cassava	250 mg kg ⁻¹	1.5 days	
<i>Fusarium fujikuroi</i>	SmF	Glucose and rice flour	1.100 mg L ⁻¹	–	Escamilla Silva et al. (1999)
<i>Fusarium fujikuroi</i>	SmF with immobilized cells	Glucose and rice flour	3.900 mg L ⁻¹	8 days	Escamilla et al. (2000)
<i>Fusarium fujikuroi</i>	SSF	Coffee husk	492 mg kg ⁻¹	7 days	Machado et al. (2002)
<i>Fusarium fujikuroi</i>	SmF-fed-batch operation	Glucose	1.680 mg L ⁻¹	4 days	Shukla et al. (2005)
<i>Fusarium moniliforme</i>	SSF	Citric pulp	5.9 g kg ⁻¹	3 days	Rodrigues et al. (2009)
<i>Fusarium fujikuroi</i>	SmF	Glucose and rice flour	1.175 mg L ⁻¹	7 days	Uthandi et al. (2010)
Mutant strains of <i>Fusarium fujikuroi</i>	SmF-fed-batch operation	Glucose and wheat gluten	600 mg L ⁻¹ of GA ₄	7 days	Lale and Gadre (2010)
<i>Fusarium proliferatum</i>	SSF	Pigeon pea pods	7.8 mg g ⁻¹	8–10 days	Satpute et al. (2010)
		Sorghum straw	5.5 mg g ⁻¹		
		Corn cobs	6.1 mg g ⁻¹		
<i>Fusarium fujikuroi</i>	SmF	Glucose and corn oil	380 mg L ⁻¹	12 days	Rios-Irbe et al. (2011)
<i>Fusarium moniliforme</i>	SmF	Glucose	15 g L ⁻¹	10 days	Rangaswamy (2012)
	SSF	Jatropha seed cake	105 mg g ⁻¹	4 days	
Mutant strain of <i>Fusarium fujikuroi</i>	SmF	Glucose	216 mg L ⁻¹ of GA ₃	10 days	Albermann et al. (2013)
<i>Fusarium fujikuroi</i>	SmF	Corn oil	360 mg L ⁻¹	12 days	Rios-iribe et al. (2016)
<i>Fusarium fujikuroi</i>	SSSF	Citric pulp	4.8 g kg ⁻¹	9 days	de Oliveira et al. (2017)

Recently, de Oliveira et al. (2017) studied the effect of different fermentation systems for GA₃ production using low agro-industrial sub-products as substrates. The authors achieved higher production using SSF (946 mg L⁻¹; 7.60 g kg⁻¹ dry citric pulp) and SSSF (331 m L⁻¹; 7.69 g kg⁻¹ dry citric pulp) than using an aqueous extract of citric pulp via SmF (236 m L⁻¹; 2.74 g kg⁻¹ dry citric pulp) in Erlenmeyer flasks. SSSF showed positive results regarding GA₃ production, and therefore, new studies with this technique may lead to its higher production with lower costs.

Factors affecting GA₃ production

Medium composition and operation systems

Whichever fermentation method is used, the chemical and physical conditions are crucial factors for the development of the microorganism, and consequently, the production of its metabolites. Among the nutrients that most affect GA₃ production, carbon and nitrogen sources are the most important. Glucose and sucrose are commonly used as carbon sources for GA₃ production, though mannitol, maltose, starch, and

glycerol are also reported (Kahlon and Malhotra 1986; Kumar and Lonsane 1990; Pastrana et al. 1995; Tomasini and Fajardo 1997; Machado et al. 2001, 2002; Rodrigues et al. 2012a).

The presence of nitrogen in the medium is important for GA production due to regulatory processes involving ammonium. However, GA₃ synthesis starts only when the exhaustion of nitrogen occurs in the medium (Bruckner et al. 1991; Escamilla Silva et al. 1999; Tudzynski 2005). Nitrogen sources such as ammonium sulfate, ammonium chloride, glycine, ammonium tartrate, corn steep liquor, and plant oil are preferred. Thus, the C:N ratio normally used to get a better yield of GA₃ is between 6:1 and 45:1, but C:N can be different, from 10:1 to 25:1 in the early stages, and from 25:1 to 200:1 in the final stages (Kumar and Lonsane 1990; Bruckner et al. 1991; Machado et al. 2001; Rodrigues et al. 2012a). This fact reveals the viability of fed-batch operations, in which it is possible to feed the medium with carbon sources during the GA₃ production process.

There are some reports about fed-batch operations for GA₃ production in the literature. Pastrana et al. (1995) studied fed-batch operation using mussel processing waste as

starting and feed mediums. With this operation system, these authors obtained an increase in biomass content as well as in the production of GA₃. Bandelier et al. (1997) successfully studied an SSF fed-batch system using wheat bran as the solid medium and cornstarch as feed solution. Shukla et al. (2007) produced GA₃ through SmF and used glucose to feed the medium. They reached 3.24 g of GA₃ total mass in fed-batch operation, almost two times greater than in the batch process (1.8 g of GA₃ total mass). Lale and Gadre (2010) reported a GA₃ production of 113 mg L⁻¹ in fed-batch fermentation after feeding the medium with glucose.

The use of agro-industrial residues as carbon or nitrogen sources was extensively reported for GA₃ production with different fermentation systems (Machado et al. 2002; Rodrigues et al. 2009; Satpute et al. 2010; Rangaswamy 2012; Rios-Irribé et al. 2016; de Oliveira et al. 2017). This practice surely provides environmentally friendly recycling, reducing the environmental impact, and enables a possible reduction of the final product cost. These residues generally present all sources of nutrients required for microorganism growth (Pandey et al. 2000; Singhania et al. 2009). Soybean, sugar beet, sweet potato, potato, and sorghum residues; wheat and rice straw; corn, rice, and soybean husks; and sugarcane and cassava bagasse can be used as substrates for GA₃ production. Processing waste from the coffee industry, fruit industries, and oil mills is also employed (Pandey et al. 2000; Soccol and Vandenberghe 2003; Singhania et al. 2009).

Physical factors

Physical factors affecting GA₃ production are temperature, pH, agitation, aeration, water activity, and/or humidity (Singhania et al. 2009; Rios-Irribé et al. 2011). The optimum temperature varies from 25 to 32 °C depending on the used strain. The optimal pH for GA₃ production is the generally employed range of 3.5–5.8 (Rodrigues et al. 2012a).

Aeration is surely necessary because the route of the biosynthesis of GAs involves a series of oxidative steps. Therefore, the microorganism's demand for oxygen may increase with the growth of mycelium. Agitation should allow efficient homogenization as well as promote the mass transfer inside the fermenter (Machado et al. 2001; Tudzynski 2005).

GA₃ separation methods

The separation and purification of biomolecules involve different steps depending on the characteristics and purity of the final commercial products. Downstream processes generally comprise higher cost techniques. Usually, the recovery of biomolecules from SmF medium is more expensive and time-consuming than from SSF or SSSF mediums. The scheme shown in Fig. 4 describes some possible ways to recover GA₃ from SSF (Bandelier et al. 1997; Satpute et al.

2010; Rodrigues et al. 2016; de Oliveira et al. 2017) and SmF (Lu et al. 1995; Ates et al. 2006; Meleigy and Khalaf 2009; Rios-Irribé et al. 2011) fermented mediums.

Generally, GA₃ recovery from SSSF and SmF starts with filtration or centrifugation steps to remove biomass and larger particles. Then, the liquid fraction is subjected to steps of adsorption, liquid–liquid extraction, or clarification. Repeated liquid–liquid partition followed by vacuum concentration is the most employed technique. For solid samples, the main step consists of extraction with solvents, but two other techniques could be used, such as supercritical fluid extraction or multiple countercurrent leaching (Kumar et al. 1991; Shukla et al. 2003). Nevertheless, these techniques can make the downstream process more expensive and involve the use of toxic solvents.

SmF is the main technique employed in industrial production of GA₃ by the fungus *Fusarium fujikuroi*. However, the GA₃ downstream industrial process is still unclear. Adsorption and liquid–liquid extraction are simple techniques that can be industrially applied to recover GA₃ from liquid extracts. The separation and concomitant purification through membranes are also applicable, but still little reported for GA₃ clarification and recovery.

Adsorption

Adsorption is commonly used in industrial purification and separation of biomolecules. The process uses an adsorbent column, which is packed with a solid resin with an affinity to the solute, to separate the desired biomolecule. Then, the biomolecule can be recovered by the loaded resin, while other components of the solution flow through the system (Magalhães et al. 2017). The biomolecule of interest is then recovered by elution with another solvent.

Adsorption columns have been employed in the recovery of GA₃. It is a simple, industrially applicable process in which it is possible to reduce energy consumption and GA₃ loss. Tang et al. (2000) compared the adsorption efficiency between medium and weak polarity (X-5, S-8, and AB-5) resins with nonpolar (D3520, D4006, and D4020) and polar (NKA-9) resins. Due to the low polarity of the GA₃ molecule, the resins with moderate polarity showed larger adsorption capacities. GA₃ recovery reached 90% using S-8 resin, and the concentration was seven times greater than before the adsorption and desorption. Another advantage is that the resin can be reused for other cycles.

Wang et al. (2008) showed similar results, in which GA₃ adsorption capacity in medium polar resins was relatively higher than in nonpolar resins and lowest in the strong polar resins. GA₃ recovery can reach 95% using methanol as eluent. Rodrigues et al. (2016) used XAD-16, C18, and active charcoal to clarify the GA₃ extract obtained after SSF. The authors reported a recovery of 35.2% using XAD-16

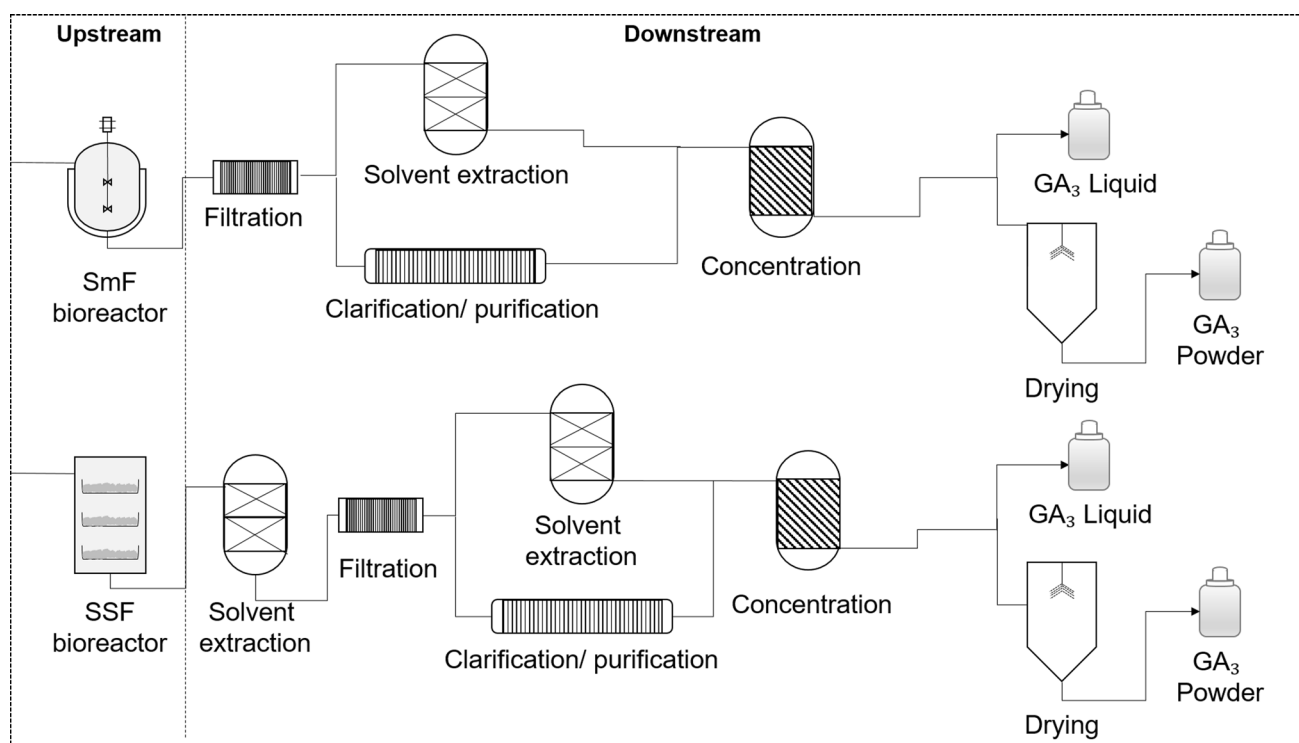


Fig. 4 Biotechnological process of gibberellic acid (GA₃) production via submerged fermentation (SmF) and solid-state fermentation (SSF), and its separation and formulation processes to obtain the bioproduct

and 42.6% using C-18. The recovery with active charcoal reached 63% of GA₃ from the extract. Active charcoal is an absorbent material that can absorb pigments from an extract and promote its clarification (Ates et al. 2006). This technique was employed and patented by Rodrigues et al. (2012b) for GA₃ fermented medium clarification.

Liquid–liquid extraction

Solvent extraction consists of passing the analyte of interest from a liquid phase (usually aqueous) to another liquid phase (usually organic) in which it has more affinity (higher solubility or chemical interaction). The most used solvent for GA₃ extraction is ethyl acetate, to which GA₃ has more affinity than water (Escamilla et al. 2000; Berrios et al. 2010). However, Uslu et al. (2014) evaluated GA₃ extraction via trydodecylamine (TDA) dissolved in three different solvents and obtained a maximum extraction of 96.37% with TDA and isoamylalcohol.

Uslu (2012) evaluated the reactive extraction for GA₃ to overcome the low extractability of most organic acids by current solvents. Reactive extraction uses an extractant to remove the acid. Long-chain aliphatic tertiary amines (anion exchange extractants) with seven to nine carbon atoms in each alkyl group are the most effective and most used extractants for carboxylic acids. The study focused on

the effects of organic diluents on the extraction of GA₃ with Aliquat 336 (A336). Diluent extractions, without A336, were less effective, and isoamylalcohol + A336 was the most effective method, with 79% of GA₃ extraction.

After extraction, a concentration step is commonly carried out to evaporate the solvent from the organic phase and recover the molecule of interest (Rodrigues 2010). In the case of GA₃, after the evaporation step, an amorphous powder or crystalline product is obtained (Bruckner et al. 1991; Shukla et al. 2003).

Membrane separation

Separation through membranes has been used for various downstream processes. However, this technique may be relatively expensive and present some operational problems. Berrios et al. (2010) found some limitations in the GA₃ separation through emulsion liquid membranes (ELMs) related to emulsion stability and water transport. Even so, 67% of GA₃ extraction from the fermented broth was reached, appearing as an alternative to the liquid–liquid extraction process.

Diafiltration and electrodialysis are two other membrane separation methods that can be employed for organic acids recovery (Magalhães et al. 2017). However, no reports were found related to GA₃ separation through diafiltration

or eletrodialysis, even with ultrafiltration techniques. The search for new industrially viable GA₃ recovery techniques is still necessary, as well as the optimization of the already used techniques, seeking the reduction of downstream costs and the reduction of GA₃ losses that usually occur.

Analysis and quantification of GA₃

During fermentation, different GAs are produced concomitantly with GA₃. The quantification and/or identification of GAs that are present in the fermented medium can be conducted via different methods, such as spectrophotometric, colorimetric, and fluorometric analysis (Berríos et al. 2004).

Spectrophotometric method

GA₃ spectrophotometric analysis at 254 nm was described by Holbrook et al. (1961). This method is simple and quick, ideal for the measurement of higher concentrations and number of samples, but it is not specific for GA₃. GA₃ is converted into gibberellic acid through broth acidification via hydrogen chloride (HCl). However, this method requires high purity of broth samples to avoid interferences during the quantification, especially when undefined fermentation mediums are used.

Berríos et al. (2004) proposed a method for GA₃ quantification that avoids the pre-treatment of the broth samples, and according to the authors, the advantage of this method is the fact that the conversion of GA₃ into gibberellic acid is lineal within 2 min of reaction when HCl is added. Their method is also based on the conversion of GA₃ to gibberellic acid through acidification, and its accuracy is above 97% for GA₃ concentration between 0.1 and 1 g L⁻¹. It is simple, fast, and reliable quantification for high amounts of samples.

Chromatographic method

Chromatographic methods are more sensitive than spectrophotometry analysis because they allow the separation and identification of the different GAs produced during fermentation. High performance liquid chromatography (HPLC) with UV detection is the most used chromatographic technique to quantify GA₃ from fermented broth (Escamilla et al. 2000; Shukla et al. 2007; Rios-iribe et al. 2016; Rodrigues et al. 2016).

However, different detectors have been employed in GA₃ quantification by liquid chromatography, such as liquid chromatography-electrospray tandem with mass spectrometry (HPLC–ESI–MS/MS) (Hao et al. 2015), HPLC coupled with fluorescence detection (HPLC–FD) (Lu et al. 2016), and ultra-performance liquid chromatography (UPLC) followed by ESI–MS/MS (Urbanová et al. 2013). Sharma et al. (2004)

studied HPLC coupled with mass spectrometry (HPLC–MS) for GA₃ detection in fermentation broth. According to them, HPLC–MS is less time-consuming and very sensitive and selective. Moreover, sample preparation is simple.

Micellar electrokinetic chromatography (MEKC) was studied as an alternative method to HPLC for GA₃ determination from fermentation broths (Nhujak et al. 2005). The authors reported as advantages of this method, for GA₃ analysis, the high accuracy and precision, shorter analysis time and there is no need of solvent extraction, only sample filtration.

Fluorescence method

Fluorometric analysis quantifies the GA₃ present in the sample through the reaction with sulfuric acid (85%), at 4 °C. The problem related to this technique is that there is no distinction between GA₃ and gibberellic acid, which may lead to quantification errors. Therefore, this method requires attention during sample preparation, and total fluorogen that is measured as GA₃ must be corrected for the amount of gibberellic acid in the sample (Kavanagh and Kuzel 1958).

Commercial products containing GA₃ as plant growth regulator

Different types of commercial formulations that are available in the market contain GA₃, or a mixture of GA₄ and GA₇. They can be obtained in liquid, soluble powder, wettable powder, tablet or water-dispersible granular forms. Some trade names of products containing GA₃ and its manufacturers are presented in Table 2.

Liquid formulations are associated with the relative lack of stability of GA₃ in the presence of water, which may lead to a short shelf life. In this way, some solvents are used to minimize GA₃ degradation, but this could result in flammable formulations that would require great care with packaging, transportation, and storage. Due to the disadvantages of liquid formulations, solid formulations seem to be a safer strategy, but they also have some drawbacks, such as dust when pouring, transferring, or measuring; the possibility of generating residues in the tank and plants; and lumps could be formed, too (Devisetty et al. 2007).

For most commercial products, GA₃ are dissolved in an alcohol solution, such as isopropanol, methanol, or ethanol, at a concentration of about 4% (w/v). Some additives and/or adjuvants are also added to increase the stability of the product, targeting a protective effect of the molecule, increasing the shelf life, or increasing nutritional factors in plants (Geary et al. 1962; Datta et al. 2006; Devisetty et al. 2006; Wang et al. 2013). Some examples of diluents, additives, or adjuvants that can be employed in a GA₃ formulation are described in Table 3.

Table 2 Different types of GA₃ products available in the international market and their manufacturers

Plant growth hormone	Formulation type	Trademarks	Producers
GA ₃	Liquid	PastureGibb®	Orion Crop Protection
		GibGro® 4LS	NuFarm Americas
		Progibb® 4%/Rizup® 4SL/Release LC	Valent BioSciences Corporation
	Soluble powder/wet-table powder	GibGro®	NuFarm Americas
		Gibb-gro®	Fertco
		Progibb® 2x/Release®/Berelex® 2x	Valent BioSciences Corporation
	Tablet	Progibb® tablet/Berelex® tablet	Valent BioSciences Corporation
	Soluble granule	Express®	Ravensdown
		Progibb® SG/Progibb® 40 SG/Activol® 40SG/Berelex® 40SG/Ryzup® 40SG/Ryzup® SmartGrass 40WSG	Valent BioSciences Corporation
		Gibb-star®	Sum Farm New Zealand Limited

Table 3 Diluents, additives, and adjuvants commonly employed for GA₃ formulations

Class	Examples
Diluents for liquid formulation	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 for solid formulation	Sorbitol, mannitol, lactose, dextrose, starch, limestone, sucrose, maltose, maltodextrin, and fructose
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
Antioxidants	Gallate (propyl, octyl and dodecyl), ascorbic acid, butylated hydroxyanisole (BHA) and butylated hydroxy-toluene (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)
UV protectant	Benzophenone-3 and ethylhexyl methoxycinnamate
Anti-caking	Silica, talc, and limestone
Binder	Maltodextrin, lecithin and polyvinylpyrrolidone

There are different formulations containing GA₃, which are patented by researchers and large companies. Some of them are presented in Table 4.

For a bioproduct formulation, the cost that will be added to its final price should be taken into account. However, the cost of additives, which is used to ensure good stability and long shelf life of the product, and the way in which the material will be packed, transported and stored must be evaluated.

Liquid or solid formulations can be applied through conventional ground or aerial applications. Spray volumes are variable depending upon the orchard or crop, the growth stage, and the climatic conditions. The concentration of the plant growth regulator will vary depending upon the type of fruit to be treated, the peculiarities of the locality, and the desired result (Devisetty et al. 2007).

Economic aspects

The demand for food by the increasing global population has sparked research on how to improve the quantity and quality of agricultural production (Liu et al. 2013). GA₃, as a plant growth regulator, plays an important economic role due to its wide range of applications, from crops to fruits, improving its production. The worldwide annual production of GA₃ is around 100 tons, with a market value of US\$ 100 million, from which approximately three quarters are used for plant production and the other quarter for malting (Rademacher 2000; Shukla et al. 2003; Rangaswamy 2012). Nowadays, GA₃ price for agricultural application ranges from US\$ 150/kg to US\$ 500/kg (prices searched in alibaba.com, accessed in 04/2018).

Table 4 Patented formulations containing GA₃

Formulation type	Strategy	Patent number	References
Powder	Extraction and concentration of the crude extract to obtain crystals of GA ₃	US 2842051	Brian et al. (1958)
Powder	Mixture of GA ₃ salts with surfactant and diammonium phosphate	US 3004845	La Pierre and Iselin (1961)
Granular	Extrusion of a mixture of salts of GA ₃ with surfactant and diammonium phosphate		
Powder	Mixture of GA ₃ powder with anhydrous salts	US 3031290	Senior and Park (1962)
Powder/liquid/paste/spray	Transformation of GA ₃ into ester and then is added the adjuvants for each type of formulation	US 3038794	Geary et al. (1962)
Liquid	Mixture of GA ₃ with organic solvent, triglycerides, esters of fatty acids and an additional plant growth hormone	WO/2000/002454A1	Rademacher et al. (1999)
Granular	Mixture of GA ₃ powder with an inert carrier, a disaccharide and a surfactant	US 6984609	Devisetty et al. (2006)
Liquid	Mixing GA ₃ powder in a blend of solvents	US 0172890	Datta et al. (2006)
Liquid	Mixture of GA ₃ powder with antioxidants, surfactants, solvent and UV protector	US 8454982	Wang et al. (2013)
Liquid	Mixture of GA ₃ , GA ₄ or GA ₇ purified with polyethylene glycol and nonionic or anionic surfactant	US 20150173365	Devisetty et al. (2015)
Liquid	Mixture of powdered gibberellins with adjuvants	US 20160360748	Pawlak (2016)

Worldwide application of GA₃ occurs in viticulture to improve fruit size. However, its application is not only limited to viticulture, Valent BioSciences Corp., one of the biggest providers of GAs, has listed more than 40 different

crops, such as fruits, vegetables and cereals, for which GA preparations can be applied, improving quality and value (Rademacher 2015). Some advantages of GA₃ application in plant development are shown in Table 5.

Table 5 Benefits of GA₃ applications in some cultures

Species	Benefits	References
Malting barley (<i>Hordeum vulgare</i>)	Increase of hydrolytic enzyme activity	MacLeod and Millar (1962)
Cherries (<i>Prunus avium</i> L.)	Increase in size, weight, and firmness of the fruits	Horvitz et al. (2002)
Black mulberry (<i>Morus nigra</i> L.)	Break of seed dormancy	Koyuncu (2005)
Cactus (<i>Trichocereus terscheckii</i>)	Seed germination	Ortega-Baes and Rojas-Aréchiga (2007)
Maize plants (<i>Zea mays</i> L.)	Overcome of salt stress	Tuna et al. (2008)
Tomato (<i>Solanum lycopersicum</i> L.)	Decrease of seed germination time and greater development in field	Balaguera-Lopez et al. (2009)
Seedless grapes (<i>Vitis vinifera</i> L.)	Increase of grape bud size	Casanova et al. (2009)
Soybean (<i>Glycine max</i>)	Amelioration of salt stress effects and restoration of the normal development of soybeans	Hamayun et al. (2010)
Sugarcane (<i>Saccharum officinarum</i> L.)	Salt tolerance	Shomeili et al. (2011)
<i>Helleborus niger</i> / <i>Helleborus x ericsmithii</i>	Promotion of flower bud growth, increase in the number and diameters of flowers	Christiaens et al. (2012)
<i>Tagetes patula</i>	Bioremediation of cadmium- and benzo[a]pyrene-contaminated soils	Sun et al. (2013)
Maize (<i>Zea mays</i> L.)	Alleviation of drought effects on maize at the vegetative phase	Akter et al. (2014)
Sunflower	GA ₃ and press mud application allowed the growth of sunflower plants in Cr-contaminated soil	Saleem et al. (2015)
Rabbiteye blueberry (<i>Vaccinium ashei</i>)	Increase of inflorescence number, and enhancement of fruit quality and plant growth	Zang et al. (2016)
Maize plants (<i>Zea mays</i> L.)	Application of GA ₃ and silicon protects corn from <i>Spo-doptera frugiperda</i> at the adult level and improves plant development	Alvarenga et al. (2017)

GA₃ is one of the best-selling and most important plant growth regulator (PGR). The advantages of its worldwide use are to improve crop production, quality and value. Because of the high investments and involved production costs, only few companies are still engaged in developing PGRs, reducing its large utilization. These companies still concentrate their investments on herbicides, insecticides and fungicides (Rademacher 2015). However, this scenario is expected to change with the search for a more sustainable agriculture, expanding the production and use of GA₃ products.

Brazil is the most important agriculture-based economy in the world. In the last harvest (2015/2017), the Brazilian planted area was estimated at 58.17 million hectares, and the estimated grain production was 238.7 million tons (Conab 2016). Moreover, Brazil is the third largest world fruit producer. In 2015, fruit production reached 41 million tons according to the Brazilian Institute of Geography and Statistics (IBGE) (Carvalho et al. 2017). The use of GA₃ in Brazilian cultivars may contribute to increase the agricultural productivity, as well as their quality, therefore, encouraging the national production and viable application of this hormone.

Future prospects

GA₃ is an important plant growth regulator from the GA family. In this review, the main topics related to GA₃ production and its commercialization and application were discussed. The main bottleneck of this plant hormone's large production is correlated with its low productivity and high costs. However, different strategies of production try to overcome these problems, such as strain improvement and alternative production strategies.

Downstream processes for GA₃ recovery are generally based on solvent extraction, adsorption and concentration methods. However, adsorption and extraction through membranes can also be applied. One of the main barriers to its application is the definition of stable liquid or solid formulations. Different GA₃ commercial products are reported and available in the world market, enabling its application in large diversity of cultivars. The search for new and low-cost techniques for GA₃ production would certainly enable its massive applicability, which would benefit the quality and productivity of different cultivars all over the world, especially in Brazil, which is one of the most important agriculture-based economies.

Author contribution statement MCC and LPSV wrote this article. CR, JO helped to write and correct the manuscript. CF and EB helped with language corrections. CRS

conceived of the study and contributed to the final text. All authors read and approved the article.

Acknowledgements This research was supported by National Council of Technological and Scientific Development (CNPq) and Coordination for the Improvement of Higher Education Personnel (CAPES).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals.

References

- Agosin E, Maureira M, Biffani V, Perez F (1997) Production of gibberellins by solid substrate cultivation of *Gibberella fujikuroi*. Adv Solid State Ferment. https://doi.org/10.1007/978-94-017-0661-2_29
- Akter N, Islam MR, Karim MA, Hossain T (2014) Alleviation of drought stress in maize by exogenous application of gibberellic acid and cytokinin. J Crop Sci Biotechnol 17:41–48. <https://doi.org/10.1007/s12892-013-0117-3>
- Albermann S, Elter T, Teubner A et al (2013) Characterization of novel mutants with an altered gibberellin spectrum in comparison to different wild-type strains of *Fusarium fujikuroi*. Appl Microbiol Biotechnol 97:7779–7790. <https://doi.org/10.1007/s00253-013-4917-7>
- Alvarenga R, Moraes JC, Auad AM et al (2017) Induction of resistance of corn plants to *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) by application of silicon and gibberellic acid. Bull Entomol Res. <https://doi.org/10.1017/S0007485316001176>
- Ates S, Ozenir S, Gökder M (2006) Effect of silicone oil on gibberellic acid production by *Gibberella fujikuroi* and *Aspergillus niger*. Appl Biochem Microbiol 42:500–501. <https://doi.org/10.1134/S0003683806050097>
- Balaguera-Lopez HE, Cardenas-Hernandez JF, Alvarez-Herrera JG (2009) Effect of gibberellic acid (GA₃) on seed germination and growth of tomato (*Solanum lycopersicum* L.). Acta Hort (ISHS) 821:141–148. <https://doi.org/10.17660/ActaHortic.2009.821.15>
- Bandelier S, Renaud R, Durand A (1997) Production of gibberellic acid by fed-batch solid state fermentation in an aseptic pilot-scale reactor. Process Biochem 32:141–145. [https://doi.org/10.1016/S0032-9592\(96\)00063-5](https://doi.org/10.1016/S0032-9592(96)00063-5)
- Berrios J, Pyle DL, Aroca G (2010) Gibberellic acid extraction from aqueous solutions and fermentation broths by using emulsion liquid membranes. J Memb Sci 348:91–98. <https://doi.org/10.1016/j.memsci.2009.10.040>
- Berríos J, Illanes A, Aroca G (2004) Spectrophotometric method for determining gibberellic acid in fermentation broths. Biotechnol Lett 26:67–70. <https://doi.org/10.1023/B:BILE.0000009463.98203.8b>
- Bömke C, Tudzynski B (2009) Diversity, regulation, and evolution of the gibberellin biosynthetic pathway in fungi compared to plants and bacteria. Phytochemistry 70:1876–1893. <https://doi.org/10.1016/j.phytochem.2009.05.020>
- Brian PW, Radley ME, Brian PW, et al (1958) Gibberellic acid compounds, and preparation and use thereof. US 2842051, 1–9

- Briggs DE (1963) Brewing industry research foundation effects of gibberellic acid on barley germination and its use in malting: a review. *J Inst Brew* 69:244–248. <https://doi.org/10.1002/j.2050-0416.1963>
- Bruckner B, Blechschmidt D, Schiller F et al (1991) The gibberellin fermentation. *Crit Rev Biotechnol* 192:163–192. <https://doi.org/10.3109/07388559109040621>
- Casanova L, Casanova R, Moret A, Agustí M (2009) The application of gibberellic acid increases berry size of ‘Emperatriz’ seedless grape. *Span J Agric Res* 7:919–927
- Chen S-Y, Kuo S-R, Chien C-T (2008) Roles of gibberellins and abscisic acid in dormancy and germination of red bayberry (*Myrica rubra*) seeds. *Tree Physiol* 28:1431–1439. <https://doi.org/10.1093/treephys/28.9.1431>
- Christiaens A, Dhooche E, Pinxteren D, Van Labeke MC (2012) Flower development and effects of a cold treatment and a supplemental gibberellic acid application on flowering of *Helleborus niger* and *Helleborus x ericsmithii*. *Sci Hortic (Amsterdam)* 136:145–151. <https://doi.org/10.1016/j.scienta.2012.01.017>
- Colebrook EH, Thomas SG, Phillips AL, Hedden P (2014) The role of gibberellin signalling in plant responses to abiotic stress. *J Exp Biol* 217:67–75. <https://doi.org/10.1242/jeb.089938>
- Conab (2016) Acompanhamento da safra brasileira de grãos. v. 9 Safra 2015/16 1–178
- Datta R, Vasek GJ, Pasieta LM (2006) Liquid formulation of a plant growth regulator. US 0172890, 1–5
- Dayan J, Voronin N, Gong F et al (2012) Leaf-induced gibberellin signaling is essential for internode elongation, cambial activity, and fiber differentiation in Tobacco stems. *Plant Cell* 24:66–79. <https://doi.org/10.1105/tpc.111.093096>
- de Oliveira J, Rodrigues C, Vandenbergh LPS et al (2017) Gibberellic acid production by different fermentation systems using citric pulp as substrate/support. *Biomed Res Int* 2017:8. <https://doi.org/10.1155/2017/5191046>
- Devisetty BN, Beach RM, Menendez RA, Warrior P (2006) Concentrated, water-soluble, granular plant growth regulator formulation and methods for use of same. US 6984609, 1–8
- Devisetty BN, Warrior P, Menendez R et al (2007) Development of fast dissolving concentrated gibberellin. *J ASTM Int* 4:3–10. <https://doi.org/10.1520/JAI100933>
- Devisetty BN, Pienaar JD, Fritts R, Venburg GD (2015) Low voc gibberellins formulations. US 20150173365, 1–12
- Economou CN, Makri A, Aggelis G et al (2010) Semi-solid state fermentation of sweet sorghum for the biotechnological production of single cell oil. *Bioresour Technol* 101:1385–1388. <https://doi.org/10.1016/j.biortech.2009.09.028>
- Escamilla Silva EM, Dendooven L, Uscanga Reynell JA et al (1999) Morphological development and gibberellin production by different strains of *Gibberella fujikuroi* in shake flasks and bioreactor. *World J Microbiol Biotechnol* 15:753–755. <https://doi.org/10.1023/A:1008976000179>
- Escamilla EM, Dendooven L, Magaña IP et al (2000) Optimization of gibberellic acid production by immobilized *Gibberella fujikuroi* mycelium in fluidized bioreactors. *J Biotechnol* 76:147–155. [https://doi.org/10.1016/S0168-1656\(99\)00182-0](https://doi.org/10.1016/S0168-1656(99)00182-0)
- Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and the control of germination. *New Phytol* 171:501–523. <https://doi.org/10.1111/j.1469-8137.2006.01787.x>
- Geary RJ, Beach V, Haber R (1962) Plant growth regulator. US 3038794, 1–4
- Gonzalez JC, Medina SC, Rodriguez A et al (2013) Production of *Trametes pubescens* laccase under submerged and semi-solid culture conditions on agro-industrial wastes. *PLoS ONE* 8:1–14. <https://doi.org/10.1371/journal.pone.0073721>
- Hamayun M, Khan SA, Khan AL et al (2010) Exogenous gibberellic acid reprograms soybean to higher growth and salt stress tolerance. *J Agric Food Chem* 58:7226–7230. <https://doi.org/10.1021/jf101221t>
- Hao YH, Zhang Z, Wang L et al (2015) Stable isotope labeling assisted liquid chromatography-electrospray tandem mass spectrometry for quantitative analysis of endogenous gibberellins. *Talanta* 144:341–348. <https://doi.org/10.1016/j.talanta.2015.06.056>
- Hedden P, Phillips AL (2000) Gibberellin metabolism: new insights revealed by the genes. *Trends Plant Sci* 5:523–530. [https://doi.org/10.1016/S1360-1385\(00\)01790-8](https://doi.org/10.1016/S1360-1385(00)01790-8)
- Hedden P, Sponsel V (2015) A century of gibberellin research. *J Plant Growth Regul* 34:740–760. <https://doi.org/10.1007/s00344-015-9546-1>
- Holbrook A, Edge W, Bailey F (1961) Spectrophotometric method for determination of gibberellic acid. *Adv Chem Ser* 28:159–167. <https://doi.org/10.1021/ba-1961-0028.ch018>
- Hollmann D, Switalski J, Geipel S, Onken U (1995) Extractive fermentation of gibberellic acid by *Gibberella fujikuroi*. *J Ferment Bioeng* 79:594–600
- Horvitz S, Godoy C, Camelo AFL et al (2002) Application of gibberellic acid to Sweetheart’s sweet cherries: effects on fruit quality at harvest and during cold storage. XXVI Int Hortic Congr Issues Adv Postharvest Hortic 628:311–316. <https://doi.org/10.17660/ActaHortic.2003.628.37>
- Kahlon SS, Malhotra S (1986) Production of gibberellic acid by fungal mycelium immobilized in sodium alginate. *Enzyme Microb Technol* 8:613–616. [https://doi.org/10.1016/0141-0229\(86\)90121-3](https://doi.org/10.1016/0141-0229(86)90121-3)
- Kavanagh F, Kuzel NR (1958) Fluorometric determination of gibberellic acid and gibberellic acids in fermentation products, commercial formulations, and purified materials. *Agric Food Chem* 6:459–463. <https://doi.org/10.1021/jf60088a006>
- Kawaide HK (2006) Biochemical and molecular analyses of gibberellin biosynthesis in fungi. *Biosci Biotechnol Biochem* 70:583–590. <https://doi.org/10.1271/bbb.70.583>
- Koyuncu F (2005) Breaking seed dormancy in black mulberry (*Morus nigra* L.) by cold stratification and exogenous application of gibberellic acid. *Acta Biol Cracoviensia Ser Bot* 47:23–26. <https://doi.org/10.17221/22/2015-JFS>
- Kumar PKR, Lonsane BK (1990) Solid state fermentation: physical and nutritional factors influencing gibberellic acid production. *Appl Microbiol Biotechnol* 34:145–148. <https://doi.org/10.1007/BF00166770>
- Kumar PKR, Sankar KU, Lonsane BK (1991) Supercritical fluid extraction from dry mouldy bran for the purification of gibberellic acid from the concomitant products produced during solid state fermentation. *Chem Eng J* 46:53–58. [https://doi.org/10.1016/0300-9467\(91\)80030-Z](https://doi.org/10.1016/0300-9467(91)80030-Z)
- La Pierre RJ, Iselin NJ (1961) Stable formulations of plant growth stimulant. US 3004845, 1–4
- Lale G, Gadre R (2010) Enhanced production of gibberellin A4 (GA4) by a mutant of *Gibberella fujikuroi* in wheat gluten medium. *J Ind Microbiol Biotechnol* 37:297–306. <https://doi.org/10.1007/s10295-009-0673-1>
- Li Y-H, Wu Y-J, Wu B et al (2011) Exogenous gibberellic acid increases the fruit weight of ‘Comte de Paris’ pineapple by enlarging flesh cells without negative effects on fruit quality. *Acta Physiol Plant* 33:1715–1722. <https://doi.org/10.1007/s11738-010-0708-2>
- Liu Y, Sun Y, He S et al (2013) Synthesis and characterization of gibberellin-chitosan conjugate for controlled-release applications. *Int J Biol Macromol* 57:213–217. <https://doi.org/10.1016/j.ijbio.2013.03.024>
- Lu XZ, Xie ZC, Kumakura M (1995) Production of gibberellic acid in *Gibberella fujikuroi* adhered onto polymeric fibrous carriers. *Process Biochem* 30:661–665. [https://doi.org/10.1016/0032-9592\(94\)00042-G](https://doi.org/10.1016/0032-9592(94)00042-G)

- Lu YH, Cao YM, Guo XF et al (2016) Determination of gibberellins using HPLC coupled with fluorescence detection. *Anal Methods* 8:1520–1526. <https://doi.org/10.1039/c5ay03133h>
- Machado CMM, Oliveira BH, Pandey A, Soccol RC (2001) Coffee husk as substrate for the production of gibberellic acid by fermentation. In: Sera T, Soccol CR, Pandey A, Roussos S (eds) *Book on coffee biotechnology and quality*. Kluwer Academic Publishers, Dordrecht, pp 401–408
- Machado CMM, Soccol CR, de Oliveira BH, Pandey A (2002) Gibberellic acid production by solid-state fermentation in coffee husk. *Appl Biochem Biotechnol* 102–103:179–191. <https://doi.org/10.1385/ABAB:102-103:1-6:179>
- MacLeod AM, Millar AS (1962) Effects of gibberellic acid on barley endosperm. *J Inst Brew* 68:322–332. <https://doi.org/10.1002/j.2050-0416.1962.tb01873.x>
- MacMillan J (2001) Occurrence of gibberellins in vascular plants, fungi, and bacteria. *J Plant Growth Regul* 20:387–442. <https://doi.org/10.1007/s003440010038>
- Magalhães AI, de Carvalho JC, Medina JDC, Soccol CR (2017) Downstream process development in biotechnological itaconic acid manufacturing. *Appl Microbiol Biotechnol* 101:1–12. <https://doi.org/10.1007/s00253-016-7972-z>
- Mai HTN, Lee KM, Choi SS (2016) Enhanced oxalic acid production from corn cob by a methanol-resistant strain of *Aspergillus niger* using semi solid-state fermentation. *Process Biochem* 51:9–15. <https://doi.org/10.1016/j.procbio.2015.11.005>
- Meleigy SA, Khalaf MA (2009) Biosynthesis of gibberellic acid from milk permeate in repeated batch operation by a mutant *Fusarium moniliforme* cells immobilized on loofa sponge. *Bioresour Technol* 100:374–379. <https://doi.org/10.1016/j.biortech.2008.06.024>
- Muñoz-Fambuena N, Mesejo C, González-Mas MC et al (2012) Gibberellic acid reduces flowering intensity in sweet orange [*Citrus sinensis* (L.) Osbeck] by repressing CiFT gene expression. *J Plant Growth Regul* 31:529–536. <https://doi.org/10.1007/s00344-012-9263-y>
- Nhujak T, Srisa-art M, Kalampakorn K et al (2005) Determination of gibberellic acid in fermentation broth and commercial products by micellar electrokinetic chromatography. *J Agric Food Chem* 53:1884–1889. <https://doi.org/10.1021/jf0484733>
- Ortega-Baes P, Rojas-Aréchiga M (2007) Seed germination of *Trichocereus terscheckii* (Cactaceae): light, temperature and gibberellic acid effects. *J Arid Environ* 69:169–176. <https://doi.org/10.1016/j.jaridenv.2006.09.009>
- Pandey A, Soccol CR, Mitchell D (2000) New developments in solid state fermentation: I-bioprocesses and products. *Process Biochem* 35:1153–1169. [https://doi.org/10.1016/S0032-9592\(00\)00152-7](https://doi.org/10.1016/S0032-9592(00)00152-7)
- Pastrana LM, Gonzalez M, Torrado A, Murado MA (1995) A fed-batch culture model for improved production of gibberellic acid from a waste medium. *Biotechnol Lett* 17:263–268. <https://doi.org/10.1007/BF01190634>
- Pawlak JA (2016) Gibberellin formulations. US 20160360748, 1–8
- Pérez FJ, Vecchiola A, Pinto M, Agosin E (1996) Gibberellic acid decomposition and its loss of biological activity in aqueous solutions. *Phytochemistry* 41:675–679. [https://doi.org/10.1016/0031-9422\(95\)00689-3](https://doi.org/10.1016/0031-9422(95)00689-3)
- Rademacher W (2000) Growth retardants: effects on gibberellin biosynthesis and other metabolic pathways. *Plant Physiol Plant Mol Biol* 51:501–531. <https://doi.org/10.1146/annurev.arpla.51.1.501>
- Rademacher W (2015) Plant growth regulators: backgrounds and uses in plant production. *J Plant Growth Regul* 34:845–872. <https://doi.org/10.1007/s00344-015-9541-6>
- Rademacher W, Schneider K-H, Kober R, et al (1999) Plant growth regulating formulations. WO/2000/002454A1
- Rangaswamy V (2012) Improved production of gibberellic acid by *Fusarium moniliforme*. *J Microbiol Res* 2:51–55. <https://doi.org/10.5923/j.microbiology.20120203.02>
- Rios-Irribé EY, Flores-Cotera LB, Chávira MMG et al (2011) Inductive effect produced by a mixture of carbon source in the production of gibberellic acid by *Gibberella fujikuroi*. *World J Microbiol Biotechnol* 27:1499–1505. <https://doi.org/10.1007/s11274-010-0603-4>
- Rios-Irribé EY, Hernández-Calderón OM, Escamilla-silva EM (2016) Kinetic analysis of the uptake of glucose and corn oil used as carbon sources in batch cultures of *Gibberella fujikuroi*. *World J Microbiol Biotechnol* 32:182. <https://doi.org/10.1007/s11274-016-2139-8>
- Rodrigues C (2010) Produção, extração e purificação de hormônio vegetal (ácido giberélico) por fermentação no estado sólido em polpa cítrica e utilização do extrato fermentado em meio de cultivo de bromélias in vitro. Universidade Federal do Paraná
- Rodrigues C, Porto L, Vandenberghe DS et al (2009) A new alternative to produce gibberellic acid by solid state fermentation. *Braz Arch Biol Technol* 52:181–188. <https://doi.org/10.1590/S1516-89132009000700023>
- Rodrigues C, Vandenberghe LPDS, de Oliveira J, Soccol CR (2012a) New perspectives of gibberellic acid production: a review. *Crit Rev Biotechnol* 32:263–273. <https://doi.org/10.3109/07388551.2011.615297>
- Rodrigues C, Oliveira J, Vandenberghe LPDS, Soccol CR (2012b) Processo de produção e purificação de ácido giberélico, seu uso e aplicações. BR n. 102012008883-5A2
- Rodrigues C, Vandenberghe LPS, Goyzueta LD, Soccol CR (2016) Production, extraction and purification of gibberellic acid by solid state fermentation using citric pulp and soy husk. *Baoj Chem* 2:8
- Saleem M, Asghar HN, Khan MY, Zahir ZA (2015) Gibberellic acid in combination with pressmud enhances the growth of sunflower and stabilizes chromium (VI) -contaminated soil. *Environ Sci Pollut Res* 22:10610–10617. <https://doi.org/10.1007/s11356-015-4275-3>
- Carvalho C de, Kist BB, Santos CE dos, et al. (2017) Brazilian fruit yearbook. Ed. Gaz. 88
- Satpute D, Sharma V, Murarkar K (2010) Solid-state fermentation for production of gibberellic acid using agricultural residues. *Int J Environment Pollut* 43:201–213. <https://doi.org/10.1504/IJEP.2010.035924>
- Selvaraj S, Murty VR (2017) Semi-solid state fermentation: a promising method for production and optimization of tannase from *Bacillus gottheilii* M2S2. *Res J Biotechnol* 12:39–48
- Senior N, Park A (1962) Dry formulations of gibberellic acid compounds. US 3031290, 1–3
- Sharma RR, Singh R (2009) Gibberellic acid influences the production of malformed and button berries, and fruit yield and quality in strawberry (*Fragaria × ananassa* Duch.). *Sci Hortic (Amsterdam)* 119:430–433. <https://doi.org/10.1016/j.scienta.2008.11.002>
- Sharma R, Iyer JP, Chakraborti AK, Banerjee UC (2004) Determination of gibberellins in fermentation broth produced by *Fusarium verticillioides* MTCC 156 by high-performance liquid chromatography tandem mass spectrometry. *Biotechnol Appl Biochem* 88:83–88
- Shomeili M, Nabipour M, Meskarbashe M, Memari HR (2011) Effects of gibberellic acid on sugarcane plants exposed to salinity under a hydroponic system. *J Afr Plant Sci* 5:609–616
- Shukla R, Srivastava A, Chand S (2003) Bioprocess strategies and recovery processes in gibberellic acid fermentation. *Biotechnol Bioprocess Eng* 8:269–278. <https://doi.org/10.1007/BF02949216>
- Shukla R, Chand S, Srivastava AK (2005) Improvement of gibberellic acid production using a model based fed-batch cultivation of

- Gibberella fujikuroi*. Process Biochem 40:2045–2050. <https://doi.org/10.1016/j.procbio.2004.07.017>
- Shukla R, Chand S, Srivastava AK (2007) Production of gibberellic acid by multiple fed-batch cultivation of *Gibberella fujikuroi*. Chem Biochem Eng 21:159–162
- Singhania RR, Patel AK, Soccol CR, Pandey A (2009) Recent advances in solid-state fermentation. Biochem Eng J 44:13–18. <https://doi.org/10.1016/j.bej.2008.10.019>
- Soccol CR, Vandenberghe LP (2003) Overview of applied solid-state fermentation in Brazil. Biochem Eng J 13:205–218. [https://doi.org/10.1016/S1369-703X\(02\)00133-X](https://doi.org/10.1016/S1369-703X(02)00133-X)
- Steffens B, Wang J, Sauter M (2006) Interactions between ethylene, gibberellin and abscisic acid regulate emergence and growth rate of adventitious roots in deepwater rice. Planta 223:604–612. <https://doi.org/10.1007/s00425-005-0111-1>
- Sun Y, Xu Y, Zhou Q et al (2013) The potential of gibberellic acid 3 (GA 3) and Tween-80 induced phytoremediation of co-contamination of Cd and Benzo [a] pyrene (B [a] P) using *Tagetes patula*. J Environ Manag 114:202–208. <https://doi.org/10.1016/j.jenvman.2012.09.018>
- Taiz L, Zeiger E (2009) Fisiologia vegetal, 4^a. Artmed, Porto Alegre
- Tang Z, Zhou R, Duan Z (2000) Separation of gibberellic acid (GA3) by macroporous adsorption resins. J Chem Technol Biotechnol 75:695–700. <https://doi.org/10.1007/s11705-008-0025-0>
- Tarkowská D, Novák O, Floková K et al (2014) Quo vadis plant hormone analysis? Planta 240:55–76. <https://doi.org/10.1007/s00425-014-2063-9>
- Tomasini A, Fajardo C (1997) Gibberellic acid production using different solid-state fermentation systems. World J Microbiol Biotechnol 13:203–206. <https://doi.org/10.1023/A:1018545932104>
- Tudzynski B (2005) Gibberellin biosynthesis in fungi: genes, enzymes, evolution, and impact on biotechnology. Appl Microbiol Biotechnol 66:597–611. <https://doi.org/10.1007/s00253-004-1805-1>
- Tuna AL, Kaya C, Dikilitas M, Higgs D (2008) The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. Environ Exp Bot 62:1–9. <https://doi.org/10.1016/j.envexpbot.2007.06.007>
- Urbanova T, Leubner-Metzger G (2016) Gibberellins and seed germination. In: Hedden P, Thomas SG (eds) The gibberellins. Annual plant reviews. Wiley, Oxford, pp 253–284
- Urbanová T, Tarkowská D, Novák O et al (2013) Analysis of gibberellins as free acids by ultra performance liquid chromatography-tandem mass spectrometry. Talanta 112:85–94. <https://doi.org/10.1016/j.talanta.2013.03.068>
- Uslu H (2012) Distribution of gibberellic acid from the aqueous phase to the organic phase. J Chem Eng Data 57:902–906. <https://doi.org/10.1021/je201337c>
- Uslu H, Datta D, Bamufleh HS (2014) Extraction equilibria of gibberellic acid by tridodecylamine dissolved in alcohols. J Chem Eng Data 59:3882–3887. <https://doi.org/10.1021/je500773w>
- Uthandi S, Karthikeyan S, Sabarinathan KG (2010) Gibberellic acid production by *Fusarium fujikuroi* SG2. J Sci Ind Res 69:211–214
- Wang R, Yang J, Shi Z, Ou L (2008) Separation of gibberellic acid (GA3) by macroporous adsorption resins. Front Chem Eng China 2:171–175. <https://doi.org/10.1007/s11705-008-0025-0>
- Wang Y, Warrior P, Lone A, et al (2013) Stable S-(+)- Absciscic acid liquid and soluble granule formulations. US 8454982, 1–8
- Wang Y, Zhao J, Lu W, Deng D (2017) Gibberellin in plant height control: old player, new story. Plant Cell Rep 36:391–398. <https://doi.org/10.1007/s00299-017-2104-5>
- Yamaguchi S (2008) Gibberellin metabolism and its regulation. Annu Rev Plant Biol 59:225–251. <https://doi.org/10.1146/annurev.arplant.59.032607.092804>
- Zang Y, Chun I, Zhang L et al (2016) Effect of gibberellic acid application on plant growth attributes, return bloom, and fruit quality of rabbiteye blueberry. Sci Hortic (Amsterdam) 200:13–18. <https://doi.org/10.1016/j.scienta.2015.12.057>

Planta is a copyright of Springer, 2018. All Rights Reserved.