

Regulation of Gibberellin Biosynthesis in *Gibberella fujikuroi*¹

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ABSTRACT

Gibberellin production by *Gibberella fujikuroi* started only after the nitrogen source was depleted and ceased upon its renewal. Nitrogen repression of gibberellin biosynthesis is not an indirect effect of the growth arrest that follows the depletion of an essential nutrient because gibberellins were not produced upon depletion of phosphate. Mycelia produced gibberellins when suspended in a glucose solution. Production ceased some time after depletion of glucose and resumed upon its readdition. Under certain conditions, the gibberellin production rate was inversely proportional to the glucose concentrations. The specific regulation of gibberellin biosynthesis by the nitrogen source imposes a revision of the concept that gibberellins are secondary metabolites whose production is triggered by imbalance or cessation of growth.

Gibberellins are natural plant hormones with practical applications in agriculture and brewing (5). Some strains of the fungus *Gibberella fujikuroi* (perfect stage of *Fusarium moniliforme*) are the industrial source of gibberellic acid (13). These strains infect rice and cause the disease known as *bakanae* in Japan (14).

More is known about the chemistry and physiology of gibberellins (9, 15) than about their biosynthesis. Gibberellin production by *Gibberella* depends upon the nature of the carbon and nitrogen sources and is stimulated by a high carbon to nitrogen ratio (2, 11, 13). Gibberellin production follows cessation of growth and exhaustion of the nitrogen source in batch cultures (2) and does not occur at low growth rates and low nitrogen concentrations (less than 65 mg·L⁻¹ in the form of glycine) in a chemostat (6). The onset of gibberellin production was attributed to the growth arrest that follows the depletion of an essential nutrient (6). This is usually assumed to be a general trait of secondary metabolites.

We have investigated the effect of nutrients on gibberellin production and have identified the nitrogen source as the critical regulator.

MATERIALS AND METHODS

The wild-type strain IMI58289 of *Gibberella fujikuroi* from the Commonwealth Mycological Institute, Kew, England,

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was grown in the dark at 30°C in 500-mL Erlenmeyer flasks with 250 mL of medium in an orbital shaker (about 200 rpm). The ICI minimal medium (high concentration of nitrogen, referred to as "high-nitrogen" minimal medium) contains 80 g·L⁻¹ of D-glucose, 4.8 g·L⁻¹ of NH₄NO₃, 5 g·L⁻¹ of KH₂PO₄, 1 g·L⁻¹ of MgSO₄·7 H₂O, and trace elements (12); the low-nitrogen version for gibberellin production contains 0.48 g·L⁻¹ of NH₄NO₃. The mannitol minimal medium differs from the ICI medium in having 60 g·L⁻¹ of D-mannitol, 0.8 g·L⁻¹ of NH₄NO₃, and 0.5 g·L⁻¹ of KH₂PO₄. The DG minimal medium contains 30 g·L⁻¹ of D-glucose, 3 g·L⁻¹ of NaNO₃, 1 g·L⁻¹ of KH₂PO₄, 0.5 g·L⁻¹ of MgSO₄·7 H₂O, 0.5 g·L⁻¹ of KCl, and trace elements (1).

Gibberellin concentrations in the media were determined by our simplified fluorometric method (8) with GA₃ (Sigma, St. Louis, MO) as a reference. A 0.2-mL aliquot of culture medium was shaken together with 0.2 mL of ethanol (96%, v/v) and 2 mL of a cooled mixture of equal volumes of sulfuric acid and 96% ethanol. After the mixture was incubated at 48°C for 30 min, the fluorescence emission at 464 nm was measured with an LS-5 Perkin-Elmer fluorimeter (excitation at 406 nm).

Glucose concentrations were determined with glucose oxidase as described by Dubois et al. (10) or with a glucose analyzer (model 27, Yellow Springs Instruments, Yellow Springs, OH). Nitrate concentrations were determined with nitrate reductase and ammonia concentrations with glutamate dehydrogenase (kit Nos. 905658 and 1112732, respectively, Boehringer Mannheim, Mannheim, Germany). Mycelia were dried for 3 h at 105°C before the determination of dry weights. In most cases, experimental repetitions were designed in such a way that results could be intercalated in the figures.

RESULTS

Specific Regulation of Gibberellin Production by the Nitrogen Supply

Gibberellins were abundantly produced in low-nitrogen minimal medium starting about 3 d after the onset of mycelial growth and accumulating in an approximately linear way for at least 3 weeks. About one-third of the glucose supply remained unused at that time. Gibberellins were not produced, and glucose was exhausted in the other minimal media (ICI and DG) with higher nitrogen concentrations.

In a mannitol minimal medium designed for gibberellin production (7), as in the traditional low-nitrogen minimal medium, gibberellin biosynthesis started upon exhaustion of

the nitrogen supply (Fig. 1); nitrate and ammonium disappeared at the same time. Except for this coincidence, the time courses of growth, nitrogen consumption, and gibberellin accumulation were different in the two media. The dry weight kept increasing after depletion of the external nitrogen source.

Addition of various nitrogen sources to cultures engaged in gibberellin production blocked further accumulation of gibberellins (Fig. 2). After nitrogen was added, the mycelial dry weight increased rapidly, and the glucose supply was exhausted within 6 d.

Results of the preceding-described experiments may sug-

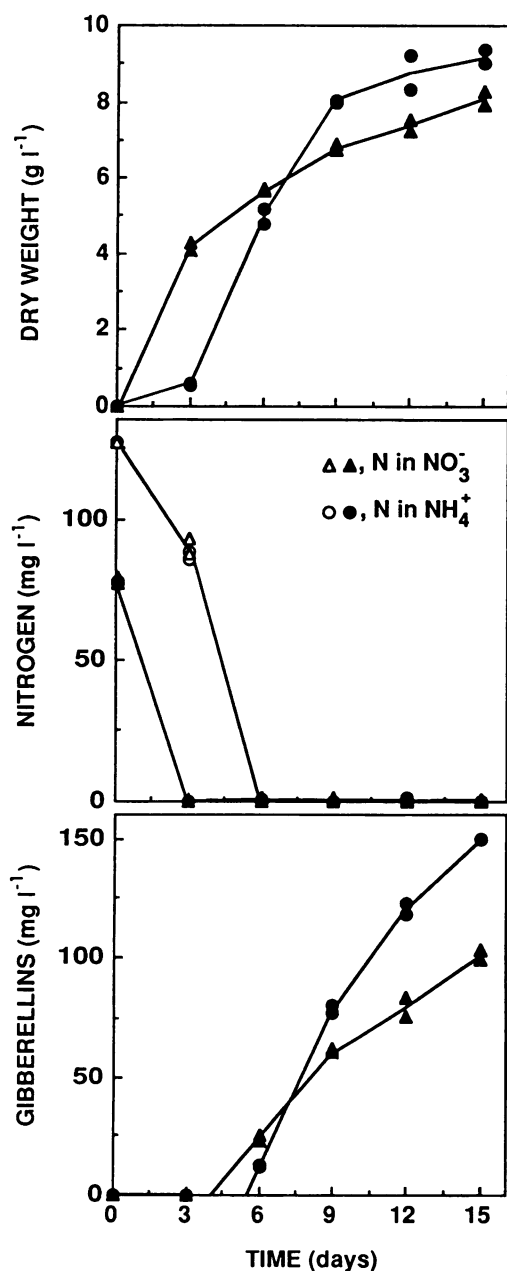


Figure 1. Mycelial dry weight and nitrogen (in the form of nitrate or ammonium) and gibberellin concentrations in cultures grown in minimal media. ●, ▲, Low nitrogen; ○, △, mannitol.

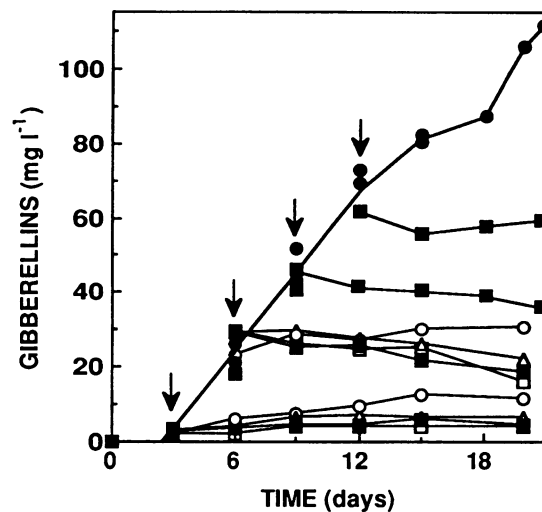


Figure 2. Inhibition of gibberellin production by nitrogen. Gibberellin concentrations in low-nitrogen minimal cultures to which a nitrogen source was added at the times indicated by the arrows or never (●). Nitrogen was added at 1.4 g·L⁻¹, i.e. 4 g·L⁻¹ of NH₄NO₃ (■) and equivalent amounts of the other sources: NH₄Cl (△); NaNO₃ (□); L-glutamine (○).

gest that gibberellin production is brought about by the unbalanced growth or cessation of growth caused by exhaustion of an essential nutrient. This explanation is refuted by the observation (Fig. 3) that gibberellins are produced under nitrogen limitation but not under phosphate limitation. The optimal initial concentration of NH₄NO₃ was 1 g·L⁻¹.

The environment, and not the mycelium, is critical for gibberellin production (Fig. 4). Cultures grown for 9 d in high- and low-nitrogen minimal media were filtered; washed mycelia and mycelium-free used media were combined in the four possible ways. Gibberellin biosynthesis occurred in used low-nitrogen medium but not in used high-nitrogen medium, irrespective of the origin of the mycelia. When mycelia from high-nitrogen cultures were transferred to used low-nitrogen medium, there was a lag, possibly needed for enzyme synthesis. Mycelia from low-nitrogen cultures maintained a residual level of gibberellin synthesis (about 12% of the previous rate) after transfer to the used high-nitrogen media. Glucose was exhausted in the high-nitrogen cultures by the 6th day; the low-nitrogen cultures contained about 40 g·L⁻¹ of glucose by the 21st day.

Effect of Carbon on Gibberellin Production

The carbons in gibberellins must evidently come from a carbon source. It is thus not surprising that carbon limitation did not allow gibberellin production. When the nitrogen concentrations were large enough to allow glucose to be exhausted first, no gibberellins were produced (Fig. 3, left).

In low-nitrogen, low-glucose media, gibberellin production stopped after a few days (Fig. 5). Addition of glucose was sufficient for gibberellin synthesis to resume at a constant rate (19.2 mg·L⁻¹·d⁻¹ in Fig. 5). This rate represented a sort of "installed capacity" for gibberellin production; when the

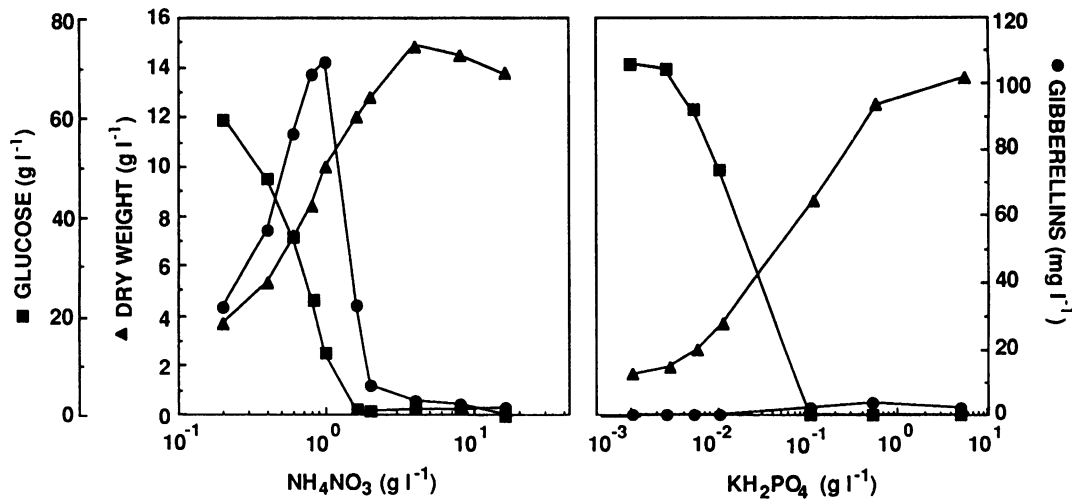


Figure 3. Gibberellin production under limitation of nitrogen or phosphate. Mycelial dry weight and glucose and gibberellin concentrations in cultures grown for 15 d in ICI minimal medium with $5 \text{ g} \cdot \text{L}^{-1}$ of KH_2PO_4 and various concentrations of NH_4NO_3 (left) or with $4.8 \text{ g} \cdot \text{L}^{-1}$ of NH_4NO_3 and various concentrations of KH_2PO_4 (right).

initial glucose and nitrogen supplies were halved, the rate was halved ($10.2 \text{ mg} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$).

A culture medium was not required for gibberellin production by previously uninduced mycelia: transfer of mycelium grown in high-nitrogen minimal medium to a glucose-phosphate solution led to lasting gibberellin production (Fig. 6).

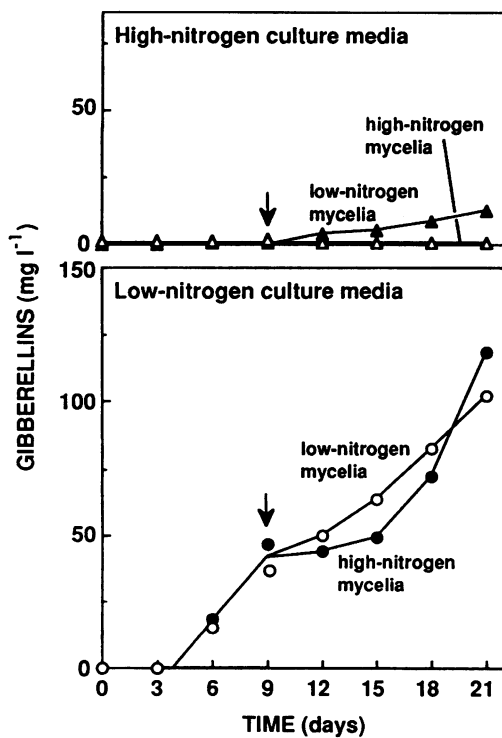


Figure 4. Exchange of mycelia and culture media. Gibberellin concentrations in high-nitrogen (top) and low-nitrogen minimal media (bottom). Nine-day-old mycelia were separated from their media, washed, and combined with their original culture media (open symbols) or with the alternative culture media (closed symbols).

The activation of gibberellin production upon transfer was not caused by transfer stress, because it did not occur in mycelia returned to the previous medium.

In experiments such as those shown in Figure 6, transfer to a glucose solution gave the the same results as transfer to a glucose-phosphate solution. Therefore, phosphate was not required for gibberellin production.

Glucose inhibited gibberellin production. High initial glucose concentrations were deleterious for the accumulation of gibberellins (Fig. 7A). The productivity of young mycelia (aged 3–6 d) was very negatively related to the glucose concentrations present in the media at the time (Fig. 7B).

DISCUSSION

Gibberellin production occurs only after depletion of the nitrogen source and is inhibited when this source is renewed. We call this phenomenon nitrogen repression, without implying any conclusion as to the underlying mechanism at the level of gene expression or enzyme activity.

Nitrogen repression is not an indirect consequence of the imbalance or the cessation of growth that accompanies depletion of an essential nutrient: gibberellin production was not induced by phosphate depletion in the presence of excess glucose and nitrogen sources. Nitrogen repression is reversible; gibberellins are produced following removal of the nitrogen source from nonproducing cultures.

A partial inhibition of gibberellin biosynthesis by nitrogen was independently observed by Brückner and Blechschmidt (4), who did not exclude the possibility that it could be a consequence of the growth arrest caused by nitrogen limitation. The appreciable gibberellin synthesis that they found in the presence of high-nitrogen concentrations suggests that their strain, a high gibberellin producer, is partially insensitive to nitrogen repression.

The absence of nitrogen is no obstacle for the production of gibberellins because these molecules contain no nitrogen atoms. Gibberellins are synthesized for many days by mycelia

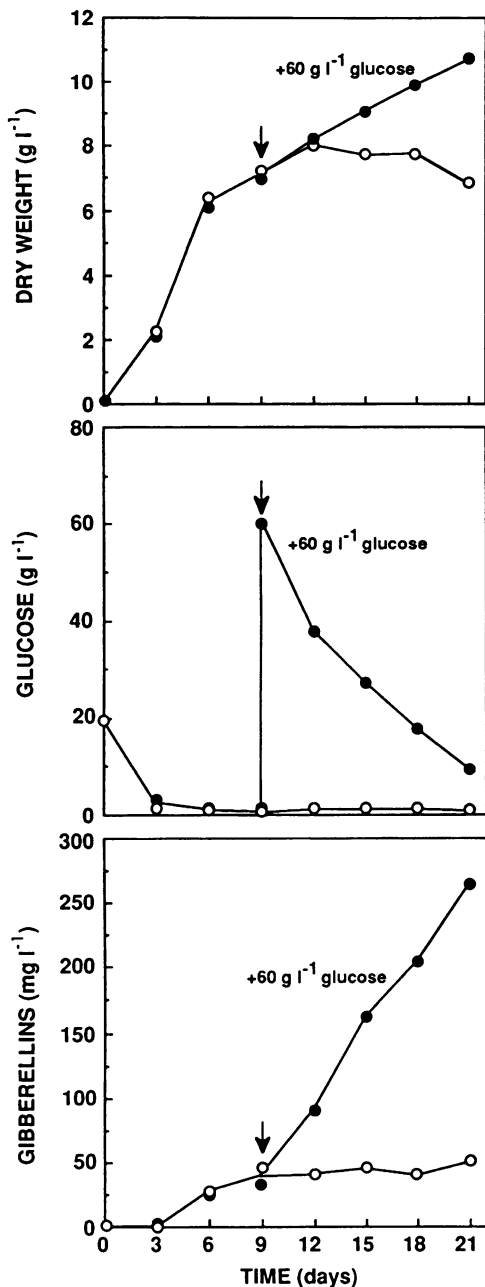


Figure 5. Arrest of gibberellin production by glucose depletion. Mycelial dry weight and glucose and gibberellin concentrations in cultures grown in ICI media with 20 g·L⁻¹ of glucose and 1 g·L⁻¹ of NO₃NH₄ (O); 60 g·L⁻¹ of glucose was added (●) to 9-d-old cultures (arrows).

suspended in a glucose solution. Gibberellin production ceases some time after depletion of the carbon source and resumes upon its renewal. The delay until cessation may be interpreted in terms of the availability of reserve substances in the cells. The increase in mycelial dry weight in the presence of glucose and the absence of a nitrogen source is due to the accumulation of carbohydrates and fats (3). Cultures abundantly supplied with glucose produce gibberellins

for a remarkably long time, at least 6 d, after depletion of glucose. The requirement of a carbon source in gibberellin production contrasts with the observation of a strong antagonism of glucose concentration and the gibberellin production rate. Our conclusions concerning the relationship of glucose and gibberellin production were largely anticipated, with different experimental approaches, by Borrow et al. (2).

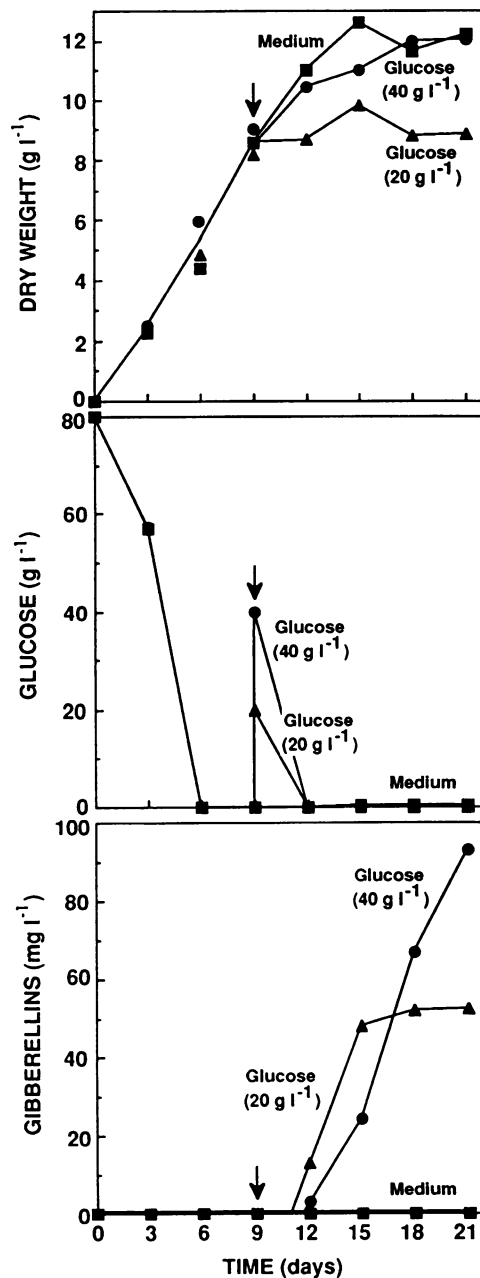


Figure 6. Gibberellin production in glucose-phosphate solution. Mycelial dry weight and glucose and gibberellin concentrations in cultures grown for 9 d in high-nitrogen minimal medium; the mycelia were then washed and transferred back to the original medium or to solutions of glucose (20 or 40 g·L⁻¹) and PO₄H₂K (5 g·L⁻¹).

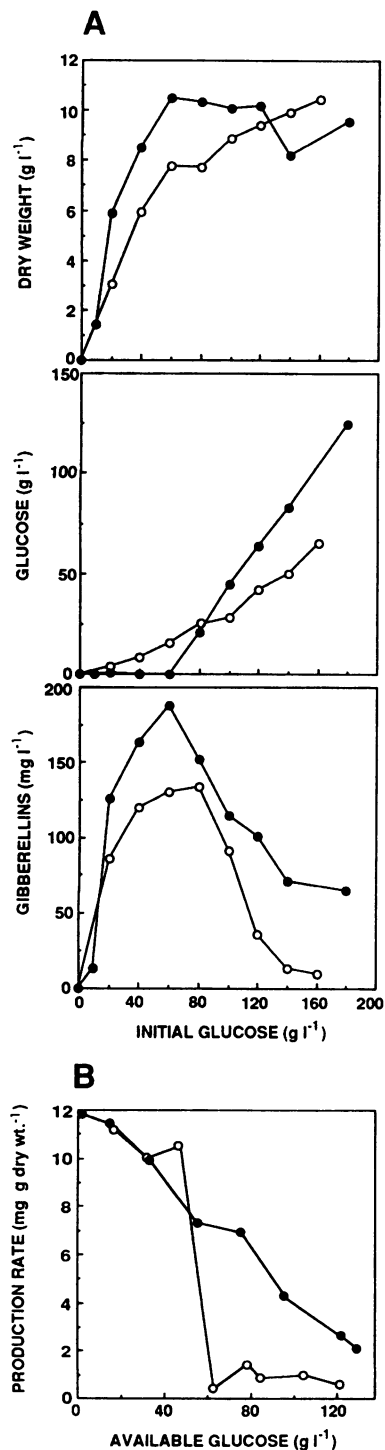


Figure 7. Inhibition of gibberellin production by glucose. A, Mycelial dry weight and glucose and gibberellin concentrations after 15 d of culture in ICI minimal media with the initial glucose concentration given on the abscissas and 1 g·NH₄NO₃ per 100 g of glucose (O) or 0.8 g·L⁻¹ of NH₄NO₃ (●). B, Gibberellin production rate in the same experiments. The mycelial dry weight and the glucose and gibberellin concentrations were measured in cultures aged 3 and 6 d. The gibberellin production rate is the ratio of the difference between the two gibberellin concentrations and the average of the two mycelial dry weights; the available glucose concentration is the average of the two glucose concentrations.

They showed that a gradual dispensation of the carbon source improves gibberellin production.

Nitrogen repression and the negative effect of high-glucose concentrations should reflect the conditions under which gibberellins are produced in the course of the infection of rice plants by *Gibberella*. A wealth of nitrogen and carbon sources in a plant should delay the onset of gibberellin production by the fungus to a later stage in plant development.

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