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Gibberellic acid production by solid substrate cultivation of *Gibberella fujikuroi* : Potential and feasibility

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Five strains of *G. fujikuroi* were screened to select *G. fujikuroi* (GF-52), a strain capable of giving consistent production of gibberellic acid (GA_3) by solid state fermentation (SSF). The comparative use of inoculum at 10% level was found to be the best against 5% and 15%. Pattern of GA_3 production was found to be the best at 5 day incubation period. Exploratory studies on the production of gibberellic acid by solid state fermentation (SSF) revealed better yield of the product as compared to that of submerged fermentation (SmF). Wheat bran, alone was the best substrate and there was no need to add any other substrates to it.

Key Words : Gibberellic acid, (GA_3), solid state fermentation, wheat bran, *Gibberella fujikuroi*, inoculum size

INTRODUCTION

Gibberellins are one of the major groups of growth promoting hormones and are secondary metabolites of the fungus, *Gibberella fujikuroi* (imperfect state *Fusarium moniliforme*). It gives higher yields of GA_3 on a variety of media. (Suruliranjan and Sarbhoy, 2000). It has been introduced as a seed application to improve seedlings establishment in seeded rice (Carlson *et al.,* 1992). Recently, gibberellins have been used for hybrid rice production (Srivastava *et al.,* 2003; Ahamad *et al.,* 2004). *F. moniliforme* produces many types of gibberellins in culture media as well as in the inoculated plants, amongst which gibberellic acid (GA_3) has received the greatest attention. GA_3 is used in parts per million levels resulting in a number of physiological effects such as elimination of dormancy in seeds, acceleration of seed germination, improvement in crop yield, marked stem elongation, promotion of fruit setting, induction of flowering in photoperiodically sensitive and cold requiring plants and in overcoming dwarfism. This resulted in a number of new plant response to gibberellins such as phenotypic reversal of genetic dwarfing, induction of flowering in long day plants and termination of several forms of dormancy. Moreover, GA_3 regulates the rate of growth and expansion of internodes (Graebe *et al.,* 1978) and is being extensively used for a variety of beneficial effects (Phinney, 1983). Ahamad and Agarwal (2004) have suggested various methods to elaborate GA_3 from different micro organisms.

Solid State Fermentation (SSF) is considered as a better technique for the production of GA_3 because of the less production cost and potentially higher yield. The production of gibberellic acid (GA_3) by fermentation is an important industry. It is selling at $ 26-37/g in the international market, depending upon its purity and potency. The SSF process is be-

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ing successfully exploited by the industry for the manufacture of fungal metabolites at better economics as compared to SmF technique. The numerous advantages associated with the SSF process led to explore the potential of SSF for the production of GA_3 (Kumar and Lonsane, 1987; Tomasini *et al.*, 1997; Prema *et al.*, 1988). Main aim of the present study is to screen out potential *Fusarium* spp. for GA_3 production, for optimize a suitable locally available cheap solid substrate for GA_3 production, effect of inoculum size of WB and pattern of GA_3 production in SSF technique. This technique is known to be highly suitable for fungal cultures. (Qian *et al.*, 1994; Kumar and Lonsane, 1990).

MATERIALS AND METHODS

Strains and culture conditions

Five strains of *G. fujikurio* (GF-52), *F. moniliforme* (FM-2451), *F. moniliforme* (FM-2193), *F. moniliforme* (FM-I) and *G. fujikuroi* (GF-1068) were used in this study (Table 1). These were maintained on potato dextrose agar (PDA) medium at 4°C and grown in Richard's broth medium. The flasks were kept on a mechanical shaker (Kuhner) with a speed of 120 rpm and constant temperature of 28°C in presence of light for developing the inoculum suspension, as light is a well known stimulator of GA_3 production (Ahmad *et al.*, 2002). The culture was allowed to grow for 10 days.

Primary inoculum development

Solid substrate i.e. wheat bran (50 g) was taken in Erlenmeyer flask. The moisture content of the substrate was adjusted to 50% using mineral salts solution of 0.2 N acidity (Sreekantiah, 1976). The flasks, each containing 50 g moist medium, were sterilized at 121°C for 2 h and cooled to ambient temperature. These were inoculated with spore suspension of the inoculum ratio of 10% of the fungal culture. The content was mixed well and the flasks were incubated at 28±1°C for 10 days. After 10 days the substrate covered by the mycelial growth was removed, mixed thoroughly and used as primary inoculum in the solid state fermentation process for GA_3 production.

GA_3 production using wheat bran

Solid substrate (wheat bran) i.e. 50 g was taken in 500 ml Erlenmeyer flask. The additives namely, substrate alone, substrate + 1% glucose, substrate + 1% gram husk, substrate + 1% molasses and substrate + 100 ppm benzyl adenine (BA) solution were added and mixed thoroughly (Table 2). The moisture content of the substrate was adjusted to 50% and flasks were sterilized. After sterilization flasks were cooled, 10% of primary inoculum was added, mixed well and allowed for fermentation (Prema *et al.*, 1988). The flasks were incubated at 28 ± 1°C for 10 days and samples were analyzed for GA_3 level. Uninoculated solid substrates served as blank. All studies were carried out in triplicate unless otherwise stated and key results were confirmed for 5-6 times for established the credibility of the data.

Extraction of GA_3 from wheat bran

For the extraction of GA_3 the fermented WB was removed from the flask, the lumps were broken and GA_3 was recovered from WB by mixing twice with equal volume of ethyl acetate for 10 minutes and subsequently filtered by using muslin cloth to get a clean extract as described by Mahadevan and Sridhar (1976). Quantification of GA_3 was done by spectrofluorodensitometer (Kumar and Lonsane, 1985). GA_3 purity was established by thin layer chromatography (Cavell *et al.*, 1967) and high performance liquid chromatography (Barendse and Van De, 1980). It is important to note that the relative quantity of solvent used in the SSF system was constant (3 ml/g moist medium).

Effect of inoculum size on GA_3 production

Studies was carried out to optimize inoculum size by using three different ratios i.e. 5%, 10% and 15%, respectively. Fully sporulated wheat bran culture was used as inoculum while the ratios were based on the weight of moist wheat bran medium (Table 3).

Pattern of GA_3 production

An experiment was conducted to reveal the pattern

of GA_3 production in 2 litre capacity of Borosil flasks for up to 7 days of incubation period in triplicates. The temperature was maintained at 28 ± 1°C.

RESULTS

Solid State Fermentation (SSF) showed better results for the production of GA_3 because of the less production cost and potentially higher yield. The results showed a marked increased in GA_3 production in SSF technique when compared to that of SmF technique. In SmF technique *G. fujikuroi*-52 recorded the highest quantity of GA_3 production (0.77 g l^{-1} g^{-1} dry weight of mycelium) followed by *F. moniliforme*-2451 (0.61 g l^{-1}) (Ahamad and Agarwal 2004). Among the various amendments evaluated, WB alone or at 1:1 ratio with rice bran, followed by WB plus 1% gram husk were found to be the best substrate i.e. 1.05, 1.04 and 1.03 g kg^{-1} DMB. WB plus 1% BA was found to be the least for GA_3 production i.e. 0.68 g kg^{-1} DMB (Table 2). Among the fungal cultures, GF-52 gave the highest GA_3 production in all six amendments followed by FM-2451 and FMS-2193, respectively. Addition of amendments such as glucose, gram husk, molasses and benzyladenine (BA) solution (100 ppm) at the rate of 1% to the substrate increased GA_3 yield considerably.

The studies on the effect of inoculum size on production of GA_3 gave interesting results. The use of inoculum at 10% level was found to be the best among the ratios employed. The extent of GA_3 production, even at the end of 3 days of incubation period, was 0.38 and 0.62 g kg^{-1} DMB with 5% and 15% inoculum ratio as compared to 10% ratio. The level of GA_3 was 1.25 g kg^{-1} DMB at the end of 4 day of incubation period as compared to 0.62 and 1.00 g kg^{-1} DMB with 5% and 15% ratios respectively. In case of 5% inoculum, the level of GA_3 increased even up to 6 days of incubation but it decreased to 1.25 and 1.00 g kg^{-1} DMB, respectively with 10% and 15% inoculum ratios if the incubation is continued beyond 4 days. Based on GA_3 formed at the end of 4 days of incubation, the rate of GA_3 production works out to be 0.006, 0.013 and 0.010 g kg^{-1} DMB respectively with 5, 10 and 15% inoculums ratio (Table 3).

Table 1. List of *Fusarium* isolates, host plant, origin and their gibberelln quantity

Strain No.	Gegraphic origin	year of isolation	Original Host	GA Content (g kg^{-1} d wt)	Production Category
1. GF-52	ITCC, New Delhi	1957	Rice seed	1.05	High
2. FM-2451	ITCC, New Delhi	1997	Wheat kernel	1.02	High
3. FMS-2193	ITCC, New Delhi	1979	Maize field	0.92	Moderate
4. GF-1068	ITCC, New Delhi	1957	Rice seed	0.68	Low
5. FM-1	Ludhiana, Punjab	1998	Rice seed	0.48	Low

Table 2 : Production of GA_3 using wheat bran (WB) as solid substrate with and without amendments

Cultures	GA_3 Yield (g Kg^{-1})						Culture Mean
	Wheat bran	W.B. + rice bran	WB + 1% glucose	WB + 1% gram husk	WB+1% molasses	WB + 1% BA	
GF-52	1.05	1.04	1.02	1.03	0.78	0.68	0.93
FM-2451	1.02	0.98	0.91	0.90	0.70	0.79	0.88
FMS-2193	0.92	0.91	0.78	0.79	0.58	0.38	0.72
GF-1068	0.68	0.69	0.75	0.72	0.40	0.32	0.59
FM-1	0.48	0.52	0.52	0.63	0.28	0.18	0.43
Substrate mean	0.83	0.82	0.79	0.81	0.54	0.47	

	S.Ed.	C.D.
Culture	0.01	0.03
Substrate	0.01	0.03
Culture X Substrate	0.03	NS

Table 3 : Effect of inoculums Size on GA_3 Production by using GF-52

Inoculums size %	GA_3 production (g Kg^{-1} DMB) Incubation periods				
	3	4	5	6	Mean
5	0.38	0.62	0.74	0.78	0.63
10	1.05	1.25	1.05	1.02	1.09
15	0.62	1.00	0.92	0.95	0.87
Mean	0.68	0.95	0.90	0.91	

Studies on the pattern of GA_3 production showed a peak on 5 days of incubation period followed by a phase of rapid decline of GA_3 production (Table 4). Thus, it was concluded that it is necessary to turn off the production of GA_3 at the time when GA_3 production is maximum. The incubation period of 5 days produced GA_3 1.23 g kg^{-1} DMB which was found to be the best for GA_3 production.

Table 4 : Pattern of GA_3 production by SSF techniques

Incubation periods (Days)	GA_3 g Kg^{-1} DMB
1	0.00
2	0.00
3	0.48
4	0.82
5	0.90
6	0.42
7	0.30

DISCUSSION

Prema *et al.,* (1988) reported that *G. fujikuroi* FT-2 followed by *B. theobromae* FT-90 recorded the maximum yield of GA_3 i.e. 1.14 g kg^{-1} DMB is SSF with 10 days incubation. Kumar and Lonsane (1987) recorded the highest GA_3 i.e. 352.0 mg kg^{-1} DMB produced by *G. fujikuroi* P-3 with 7 days incubation. The highest concentration reported in solid state cultures was 1.2 g GA_3 kg^{-1} dry cultures in WB medium (Kumar and Lonsane 1990). Similar trend was also observed in the present study. However, Qian *et al.,* (1994) produced 18.7 to 19.3 mg g^{-1} dry weight of mycelium of GA_3 in MB medium with *F. moniliforme* M-7121.

GA_3 yield was maximized with the addition of gram husk as amendment to all the substrates when compared to other amendments. When tried with yield was 0.85 g kg^{-1} of rice bran and with bagasse it was 0.98 g kg^{-1}. The reason might be nutrient content of gram husk. Moreover, bigger particles size of gram husk will allow lot of air spaces leading to better O_2 diffusion and efficient removal of CO_2 and other volatile products. Even though molasses are also rich in nutrients, GA_3 yield was lower when compared to gram husk. Addition of molasses resulted in compact mass formation after sterilization making difficult for easy exchange of gases.

Among the various amendments evaluated, WB alone or at 1:1 ratio with rice bran was found to be the best substrate for GA_3 production. Enrichment of WB with BA or molasses resulted in lower productivity. But with 1% glucose and gram husk, a slight increase in the rate of GA_3 production was observed. In the present study, GF-52 gave maximum GA_3 1.05 g l^{-1} followed by FM-2451. The results of the present studies are in accordance with the findings of Qian *et al.,* (1994) with the production of 1.23 g kg^{-1} DMB where as Kumar and Lonsane (1987) produced 352.0 mg kg^{-1} DMB.

The production of GA_3 under the SSF process by *G. fujikuroi* FT-2 at flask and large scale level are found to be variable and inconsistent. In most of the cases, the yield was ~ 1g/kg DMB (Dry Moldy Bran), even at 96 and 150 tray levels (Ahamad *et al.,* 1987). But varied between 0.28 and 1.23 g of GA_3 kg^{-1} of DMB (Sreekantiah, 1976). *G. fujikuroi* P-3 gave consistent production of GA_3 under SSF process, but yield was ~ 0.35 g kg^{-1} of DMB in basal WB medium, selected arbitrarily (Kumar and Lonsane 1988, 1987b). After standardization of various physical and nutritional parameters, an optimum medium was developed and it consistently gave 0.986-1.115 g of GA_3 kg^{-1} of DMB in flask level experiments (Kumar and Lousane, 1987).

Research performed by Kumar and Lonsane (1976, 1990, 1987a) and Agosin *et al.,* (1995) show that gibberellic acid can be produced in high quantities by SSF on wheat bran. Ahamad *et al.* (2005) reported that use of coarse wheat bran (0.3-0.4 cm) resulted in 2.5 fold increase GA_3 yield. Even the yields of GA_3 from most of the strains are low to moderate, it could be used for optimization of process by selection, mutation, and molecular tachniques to improve GA_3 in higher yields and at economic

costs. The GA_3 yield was consistent in all the selected fungal cultures which indicated stability of fungal cultures for GA_3 production.

REFERENCES

Ahamad, S. and Agarwal, D. K. 2004. Microbial production of Giberellin : An Overview. In : *Biological Diversity : Current Trends* edited by S. K. Gauitam, Y. K.Bansal and A. K. Pandey. Published by Shree publishers & Distributors, New Delihi pp 158-172.

Ahamad, S., Agarwasl, D. K. and Chauhan, S. S 2005 Production of gibberellic acid by solid state fermentation of *Gibberella fujikuroi:* consistent and improved yield. *Ind. J. Microbiol. (In press).*

Ahmed, S. Y., Lonsane, B. K., Ghildayal, N. P. and Ramkrishn, S. V. 1987. Biotechnol. Tech. **1** : 97-107.

Ahamad, S., Narain, U. Agarwasl, D. K. and Chauhan, S. S. 2002a. Effect of Temperature, pH, Light and Incubation periods on growth, Sporulation, biomass and Gibberellic acid production. *Ann. Pl. Protec. Sci.* **10** : 243-248.

Ahamad, S., Sarbhoy, A. K. and Srivastava, A. C. 2004. Effect of gibberellic acid produced with *Gibberella fujjkuroi* on CMS lines of Rice plant. *Ann. Pl. Protec. Sci.* **12** : 80-85

Agosin, E., Maureira, M., Biffani, V and Perez, F. 1995. Production of gibberellins by Solid substrate cultivation of *Gibberella fujikuroi.* II International Workshop on solid state fermentation, February 27-18. Montpellier, France, p. 28.

Barendse, G.W.M. and Van De, Werken. 1980. High Performance Liquid Chromatography. *J. Chromato.* **198** : 449-455.

Carlson, R. D., Chang, N., Black-Schaefer, C. and Fugiel, J. A. 1992. Efficacy of release seed treatment in California rice production systems. In : Proceedings of the 4th Annual Conference of the Western plant growth regulator Society, Sacramento, January 22-23. pp. 109-129.

Cavell, B. D., Macmillan, J., Pryce, R.J., and Sheppard, A. C. 1980. Thin layer Chromatography. *Phytochem.* **6** : 867-874.

Graebe, J. E. and Ropers, H. 1978. Gibberellins. In : Letham, D.S. *et al.* (eds.) *Phytohormones and related compounds-a compprehensive treatise.* Amsterdam : Elsevier/North Holland Biomedical Press. PP. 107-129.

Kumar, P. K. R. and Lonsane, B. K. 1985a. In 26th Annual Conference of the Association of Microbiologist of India. Abst. IND., 0-5, p 110, AMI, Madras.

Kumar, P. K. R. and Lonsane, B. K. 1987a. Potential fed batch in solid state fermentation for the production of gibberellin. *Biotechnol.Lett/.* **9** : 179-182.

Kumar, P. K. R. and Lonsane, B. K. 1987a. Potential fed batch in solid state fermentation for the production of gibberellin. *Biotechnol.* Lett., **9** : 179-182.

Kumar, P. K. R. and Lonsane, B. K. 1987b. Gibberellic acid by solid state fermentation. consistent and improved yields. *Biotechnol. Bioeng.*, **30** : 267-271.

Kumar, P. K. R. and Lonsane, B. K. 1987c. Extraction of Gibberellic acid from mouldy bran produced under solid state fermentation. *Pro. Biochem.*, **22** : 139-143.

Kumar, P. K. R. and Lonsane, B. K. 1990. Solid state fermentation : physical and nutritional factors influencing Gibberellic acid production. *Microbiol. Biotechnol,* **4** : 145-148

Mahadevan, A. and Sridhar R. 1976. *Methods in Physiological Plant Pathology.* Sivakami publications. Madras. p. 316.

Phinney, B. O. 1983. The history of the gibberellins. In: Crozier, A. (ed.). *The biochemistry and physiology of gibberellin.* New York: Praeger Press. PP. 19-27.

Prema, P., Thakur, M. S., Prapulla, S. G., Ramkrishna, S. V. and Lonsane, B. K. 1988. Production of Gibberellic acid by solid state fermentation. *Indian. J. Micribiol.* **28** : 78-81

Qian, X. M., Dupreez, J. C. and Kilian. S. G. 1994. Factors affecting gibberellic acid production by *Fusarium* moniliforme in solid state cultivation on starch. *World J. Microbiol. Biotechnol.* **10** : 93-99.

Sreekantiah K. R. 1976. *Studies on pectin degrading strain of fungi.* Ph.D. Thesis, Mysore University, Mysore, India.

Srivastava, A. C., Ahamad, S., Agarwal, D. K. and Sarbhoy, A. K. 2003. Screening of potential gibberellin producting *Fusarium* strains for the hybrid rice production. *Food, Agriculture & Environment.* **1** : 250-253.

Surulirajan, M. and Sarbhoy, A. K. 2000. Gibberellic acid production by *Fusarium moniliforme. J. Mycopathol. Res.* **38** : 101-104.

Tomasini, A., Fajarado, C., and Barrios-Conzalez, J. 1997. Gibberellic acid production using different solid state fermentation. *World Jr. of Microbiol* Biotechnol. **13** : 203-206.

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