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RESEARCH ARTICLE

# Screening and development of effective mutants of *Fusarium fujikuroi* (*Gibberella fujikuroi*)

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*Introduction*

Gibberellins (GAs) are a group of diterpenoid acids with ent-gibberellane skeleton that function as plant growth regulators influencing a range of devel- opmental processes in higher plants including stem elongation, germination, dormancy, flowering, sex expression, enzyme induction, leaf and fruit senes- cence (Graebe and Ropers, 1978). Gibberellic acid is a high value, industrially important biochemical, sell- ing high rate in the international market depending on the purity and potency. Gibberellic acid also has potential application in improving seedling establish- ment and seed production in hybrid rice (Carlson *et al*., 1992). In India up to 1970's research on gibberel- lic acid mainly focused on the isolation and identifi- cation of GAs from plant sources. Studies conducted at CFTRI, Mysore in 1970s ended with a GA3 yield of 0.40 - 0.45 g per litre in submerged fermentation pro- cess (SmF) even after optimizing the culture condition parameters. The efficient strain selection and usage plays key role to make the gibberellic acid production economically viable. The strain improvement strategy for increased GA production can be achieved by mu- tation and protoplast fusion in filamentous fungi like F.fujikuroi. In the present work, we have screened and developed a procedure to obtain effective F.fujikuroi mutants through physical and chemical mutagenesis for increased gibberellic acid production.

Key words: *Fusarium fujikuroi,* Mutation, Biogibberellic acid

*Methods*

Screening of microbial cultures and Mutation / Mutagenesis appears to be a tangible method for de- veloping strains with improved beneficial traits. For enhancing gibberellic acid production, the screening followed by physical and chemical mutagenesis tech- niques is applied. *Screening Fusarium isolates for* $GA_3$ *production*

Czapek-Dox liquid medium dispensed in 100 ml quantities in 500 ml Erlenmeyer flasks was employed and the cultures listed in table 1 were inoculated at the ratio of 5 per cent (v/v) The flasks were incubated at 30±1°C for 7 d on rotary shaker (Environ shaker 3597-IL- BGM, Lab line instruments, Illinois) at 150 rpm. Samples were removed at the end of 7 d of fermentation for es- timation of $GA_3$.

***Collection of Fusarium fujikuroi spores***

*F. fujikuroi* cultures were grown on a carbon limited agar medium for 5 d at 30°C. Micro conidia were harvested by washing the sporulated mycelia with sterile distilled water and separated by passage through filter paper. Number of conidia per ml was quantified by

haemocytometer and viable counts were made by plating in nutrient medium and enumerating CFU. Freshly collected spore suspensions with $10^4$ conidia per ml was used for mutagenesis.

*Physical mutagenesis with UV for increased $GA_3$ production*

The selected *F. fujikuroi* cultures were grown on a carbon limited agar medium for 5 d at 30°C. Micro conidia were harvested by washing the sporulated mycelia with sterile distilled water and separated by passage through filter paper. Freshly collected spore suspensions with $10^4$ conidia per ml were exposed to UV light. Optimum dose required to get maximum mutants was arrived by exposing the organism for dif- ferent periods of time (30, 60, 90, 120 and 150 min) in
different distances (10, 20, 30, 40, 50 cm) from the UV source. UV exposure was followed by 5 hours incuba- tion in light for photo reactivation. The dose, which gave one per cent survival, was selected for the in- duction of mutants. The stable mutants were selected based on the consistent expression of the phenotyp- ic character upto six generations and maintained on PDA slants for experimental purposes.

*Chemical mutagenesis with NTG for increased $GA_3$ production*

Freshly collected spore suspensions of select- ed *Fusarium fujikuroi* cultures having $10^4$ conidia $ml^{-1}$ were suspended in sterile testubes with one ml of different concentrations of N-methyl-N-nitro-N-ni- trosoguanidine. Optimum dose required to get maxi- mum mutants was arrived by exposing the fungus to various concentrations of mutagen *viz*., 250, 500, 750, 1000 and 2000 ppm for different periods of time (15, 30, 45, 60 and 90 min). Treatments were stopped by diluting and washing the mixture in sterile distilled water. The concentration, which allows one per cent survival, was selected for the induction of mutants. Suitable untreated control was maintained. The sta- ble mutants were selected based on the consistent expression of the phenotypic character upto six gen- erations and maintained on PDA slants for experi- mental purposes. Adequate protection during NTG treatment was ensured by using masks, gloves and disposable materials. Treated glassware was boiled in 1*N* NaOH solution for 20 min. under a hood in order to inactivate NTG before washing or discarding.

*Results and discussion*

It is observed from the results that all the cul- tures were able to produce $GA_3$, however wide vari- ations were seen among the cultures with regard to yield. The isolate SG2 produced the maximum $GA_3$ cm between the UV source and the spore suspension of *Fusarium fujikuroi* SG-2.This optimized UV irradi- ation dosage was used for the induction of mutants in *Fusarium fujikuroi* SG20B and *Gibberella fujikuroi* IMI 58289. The dosage survival curve explains 0.21per cent was ideal for the selection of mutants.

The spore suspension of *Fusarium fujikuroi* SG-2 was exposed to different concentrations of NTG rang- ing from 250 - 2000 ppm for different periods. Results showed that the increased period of exposure cou- pled with the increased concentrations of NTG had a direct correlation with increased mortality rate (Table 4 and 5). The concentrations ranging from 250-750 ppm was least effective in reducing the survival rate to the desired level required for the induction of mu- tants. The least survival of 0.30 per cent was recorded at a concentration of 2000 ppm coupled with an expo- sure period of 90 minutes.

$GA_3$ is not produced in India and hence millions of rupees are involved in its import. Because of the high cost the use of gibberellic acid is at present limit- ed to certain high value crops. Owing mainly to its use in viticulture and malting, the demand for $GA_3$ in India is more than 0.5 ton/annum and inspite of the con- straint, the demand is increasing day by day. Any at- tempt to reduce the cost of production of $GA_3$ will be of immense value because reduction in cost will lead to its wider application to a variety of crops. It was stressed by Kahlon and Malhotra (1986) that much can be gained by producing $GA_3$ indigenously in India.

Morphological and biochemical mutants of *G. fujikuroi* have been isolated after treatment with dif- ferent chemical and physical mutagens (Bearder *et al*., 1974). Not surprisingly, these efforts have been concentrated largely on mutants affecting gibberellin production. In the present investigation the least sur-(542.00 mg l ) followed by SG20B

(474.66 mg l ) and IMI 58289 (388.66 mg $l^{-1}$). The lowest GA yield was observed in *Fusarium* spp. GSG4 (96.83 mg $l^{-1}$). In gen- eral, final pH of the medium and the fungal biomass ranged between 2.81 to 5.18 and 3.14 to 6.50 g $l^{-1}$ re- spectively.

The spore survival of *Fusarium fujikuroi* SG-2 af- ter different periods of exposure to UV light was as- sessed and the results are furnished in Table 2 and 3. The increased period of exposure and the minimum distance between the UV source and the parent to be mutated is directly correlated with the mortality rate. The least survival of 0.21 per cent was observed at an exposure period of 150 min. with a distance of 10 vival of 0.21 per cent was observed at an exposure period of 150 min. with a distance of 10cm between the UV source and the spore suspension of *Fusarium fujikuroi* SG-2. Twenty four mutant clones were ob- tained from *Fusarium fujikuroi* SG2, *Fusarium fujikuroi* SG20B and *Gibberella fujikuroi* IMI 58289. Many of the mutants exceeded the parent strains in terms of gibberellic acid yield.

N-methyl-N'-nitro-N-nitrosoguanidine (nitro- soguanidine) has been extensively used for the in- duction of mutations in many organisms (Gichner and Velminsky, 1982). In the present study, the concentra- tions ranging from 250-750 ppm was least effective

Table 1. Screening Fusarium isolates for $GA_3$ production under SmF

| Isolates | GA mg $l^{-1}$ | Fungal biomass * g $l^{-1}$ | Final pH of the medium |
|---|---|---|---|
| GSG -1 | 245.00 | 4.32 | 3.20 |
| GSG-2 | 174.66 | 5.75 | 5.10 |
| GSG -3 | 287.00 | 4.21 | 3.64 |
| GSG-4 | 96.83 | 5.40 | 4.75 |
| GSG-5 | 208.16 | 3.14 | 2.81 |
| NCIM 1019 | 385.00 | 5.42 | 3.24 |
| IMI 58289 | 388.66 | 5.66 | 4.16 |
| ATCC 1464 | 381.00 | 6.45 | 2.98 |
| PAT | 227.16 | 4.25 | 4.05 |
| SG 2 | 542.00 | 6.50 | 3.15 |
| SG 11 | 252.66 | 4.37 | 3.28 |
| SG 12 | 263.00 | 4.60 | 3.63 |
| SG 18 | 287.50 | 3.50 | 3.81 |
| SG 19 | 360.00 | 5.46 | 4.20 |
| SG 20B | 474.66 | 3.42 | 3.95 |
| SG 20C | 225.00 | 5.19 | 4.46 |

| | | | |
|---|---|---|---|
| SG 20D | 230.00 | 5.16 | 4.58 |
| FC1 | 103.53 | 4.24 | 4.85 |
| FF | 204.66 | 5.66 | 5.14 |
| FM3 | 144.16 | 5.46 | 5.18 |
| FM4 | 220.66 | 5.16 | 5.15 |
| FS | 135.83 | 4.84 | 4.48 |
| SEd | 11.35 | 0.20 | |
| CD (p = 0.05) | 22.88 | 0.41 | |

Table 2. Induction of mutants through UV irradiation

| Distance from UV source (cm) | Survival of Fusarium fujikuroi after exposure to different periods (cfu x $10^2$ $ml^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | 30 min | 60 min | 90 min | 120 min | 150 min | Mean |
| 10 | 408.66 | 242.33 | 106.66 | 4.00 | 1.33 | 134.60 |
| 20 | 442.33 | 357.33 | 110.00 | 7.33 | 4.66 | 184.33 |
| 30 | 475.00 | 378.66 | 153.33 | 14.66 | 7.00 | 205.73 |
| 40 | 512.33 | 384.33 | 196.66 | 23.66 | 16.33 | 226.66 |
| 50 | 563.33 | 471.00 | 208.33 | 31.33 | 28.00 | 260.40 |
| Control | 634.00 | 614.33 | 642.00 | 608.66 | 602.00 | 622.20 |
| Mean | 505.11 | 408.00 | 221.16 | 114.94 | 109.89 | - |
| SEd | 19.99 | 16.59 | 12.16 | 10.16 | 10.04 | |
| CD (P=0.05) | 43.57 | 36.16 | 26.51 | 22.15 | 21.89 | |

Table 5. Per cent survival after exposure to NTG

| Dosage (ppm) | Per cent survival of Fusarium fujikuroi after different periods of exposure | | | | | |
|---|---|---|---|---|---|---|
| | 15 min | 30 min | 45 min | 60 min | 90 min | Mean |
| 250 | 90.98 | 80.37 | 63.67 | 45.25 | 32.98 | 62.65 |
| 500 | 85.92 | 67.40 | 52.16 | 37.59 | 29.43 | 54.50 |
| 750 | 75.82 | 59.26 | 39.93 | 32.48 | 21.28 | 45.75 |
| 1000 | 61.00 | 40.00 | 22.66 | 14.23 | 0.42 | 27.66 |
| 2000 | 32.12 | 20.00 | 13.66 | 6.20 | 0.32 | 14.46 |
| Control | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Mean | 74.31 | 61.17 | 48.68 | 39.29 | 30.73 | - |

in reducing the survival rate to the desired level re- quired for the induction of mutants. The least surviv- al of 0.30 per cent was recorded at a concentration of 2000 ppm coupled with an exposure period of 90 minutes. The results of the studies are comparable to the findings observed earlier (Avalos *et al*., 1985).

Interestingly, nitrosoguanidine survivors upon further characterization resulted in five auxotrop- hic mutants, five carbendazim resistant mutants, six

copper sulphate resistance mutants, four albino mu- tants and two *nit* mutants among the morphological mutants screened. Screening all the biochemically characterized mutants

revealed higher $GA_3$ yield in fungicide resistant mutants compared to other mor- phological mutants and wild type.

*Conclusions*

In the present study, the non pathogenic higher gibberellic acid producing strains were isolated. A effi- cient protocol to enhance the gibberellic acid produc-tion from the isolated *F.fujikuroi* cultures were also standardized.

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