

Production of Gibberellin by Fungal Strains of Gibberella sp

李延卿、陳鴻章

E-mail: 8809446@mail.dyu.edu.tw

ABSTRACT

The research studied the production of gibberellin by filamentous fungal strains : Gibberella fujikuroi CCRC 32512 and CCRC 32513. By changing only one variable on each trial, it examined the effect on the production of gibberellin in relation to variables such as mycelium density, homogenization time, inoculum ratio, fermentation volume, shaker velocity and growth temperature, etc. In relation to mycelium density, the greatest production of 130.31 mg/L and 140.31 mg/L respectively occurs when 0.6 g each of Gibberella fujikuroi CCRC 32512 and CCRC 32513 were scraped and put into homogenization for 240 sec. As to the inoculum ratio, the gibberellin grew the most when 4% (v/v) of suspending filamentous fungal strain were cultivated in the 50 mL medium for fermentation. The production of gibberellin by both Gibberella fujikuroi CCRC 32512 and CCRC 32513 reached the respective optimum of 76 mg/L and 98 mg/L in the fermentation volume of 50 mL. Shaker velocity has a significance on the production of gibberellin too. For example, under 250 rpm, Gibberella fujikuroi CCRC 32512 and CCRC 32513 each produces 96 mg/L and 145 mg/L of gibberellin, 1.7 and 2.0 times of that under 100 rpm. In terms of growth temperature, the production of gibberellin was the most desirable under the temperature of 31°C for both strains with respective yields of 74 mg/L and 118 mg/L. Through the result of the above experiments, we found G. fujikuroi CCRC 32513 performed more outstandingly than G. fujikuroi CCRC 32512 in respect of fermentation volume, shaker velocity and growth temperature. Thus, G. fujikuroi CCRC 32513 was selected to continue the studies of the effect on the production of gibberellin in relation to variables such as source and concentration of carbon and nitrogen and the amount of buffering agent, etc. The relevance between the production of gibberellin and buffering agents showed the addition of 0.5 g/L sodium dihydrophosphate and 1.0 g/L di-potassium hydrophosphate promoted the most the production of gibberellin, while sucrose served as the best carbon source at the amount of 40 g/L. In relation to nitrogen source, G. fujikuroi CCRC 32513 was able to generate the most gibberellin with peptone being its best nitrogen source at the amount of 12 g/L. In the optimal combination of culture media, the production of gibberellin by G. fujikuroi CCRC 32513 reached as high as 289 mg/L 7 days later, 3.1 times of that in the basal medium. By means of orthogonal array design L16(2¹⁵), the research also examined the major effect of each factor and the interaction effect among the factors on the production of gibberellin by G. fujikuroi CCRC 32513. The first L16(2¹⁵) matrix showed the major factors that affected the production of gibberellin included the concentration of carbon source, nitrogen source and the cultural time, Other factors did not have a significant influence on the production. The factors such as concentration of carbon source, nitrogen source and the cultural time in high levels were further set in the second L16(2¹⁵) design, and the analysis showed more gibberellin was generated when G. fujikuroi CCRC 32513 was cultured in the media of 40 g/L sucrose, 12 g/L peptone for 4 days than in the media of 60 g/L sucrose and 18 g/L peptone for 8 days. At last, with the rest factors fixed at the optimal condition obtained from the tests in which only one factor was changed each time, the setting of the above mentioned factors in low levels in the third L16(2¹⁵) matrix showed that the most favorable range of carbon and nitrogen concentration were 30—40 g/L and 9—12 g/L respectively, and the optimal cultural time was 4 days. The experiment showed the combination led to a higher production of 348 mg/L, and the major effect of each factor and the interaction among factors have a significant influence on the production of gibberellin. Key words : filamentous fungal strains, gibberellins, orthogonal array design

Keywords : filamentous fungal strains ; gibberellins ; orthogonal array design

Table of Contents

封面內頁 簽名頁 授權書	iii	中文摘要	v	Abstract	vii	誌謝	x	目錄	xi	圖目錄	xv	表目錄	xviii	第一章 緒論	1	1.1 研究背景	1	1.2 研究之重要性	2	1.3 研究目的	2	第二章 文獻回顧	3	2.1 激博素發展歷史	3	2.2 激博素之結構及物化特性	6	2.3 激博素之生理功用	9	2.3.1 種子萌芽	10	2.3.2 激博素與種子休眠之關係	11	2.3.3 莖之生長	12	2.3.4 根之生長	13	2.3.5 葉之生長	13	2.3.6 果實發育	14	2.3.7 開花作用	14	2.3.8 誘發植物糊粉層中 α-amylase之合成	16	2.4 激博素的生合成及調節機制	17	2.5 激博素之應用	22	2.6 激博素的生產菌	23	2.7 影響真菌生產激博素之因子	24	2.7.1 菌絲密度	24	2.7.2 發酵容積	27	2.7.3 pH值	28	2.7.4 培養溫度	29	2.7.5 碳源種類	30	2.7.6 碳源濃度	31	2.7.7 氮源種類	31	2.7.8 氮源濃度	33	2.8 激博素之回收與純化	34	2.9 直交矩陣設計之介紹	36	2.9.1 直交矩陣之概念	36	2.9.2 種類與研究範圍	37	2.9.3 L16(2 ¹⁵) 直交排列法之構成	37	2.9.4 L16(2 ¹⁵) 直交排列法之因子配置	38	2.9.5 因子配置之方法	41	2.9.5.1 成份運算	41	2.9.5.2 線點圖	42	第三章 實驗材料與方法	43	3.1 實驗設備	43	3.2 實驗材料	44	3.2.1 菌株來源	44	3.2.2 培養基之組成	44	3.2.3 重要藥品、標準品及分析管柱	45	3.3 實驗方法	46	3.3.1 菌種保存	
--------------	-----	------	---	----------	-----	----	---	----	----	-----	----	-----	-------	--------	---	----------	---	------------	---	----------	---	----------	---	-------------	---	-----------------	---	--------------	---	------------	----	-------------------	----	------------	----	------------	----	------------	----	------------	----	------------	----	-----------------------------	----	------------------	----	------------	----	-------------	----	------------------	----	------------	----	------------	----	-----------	----	------------	----	------------	----	------------	----	------------	----	------------	----	---------------	----	---------------	----	---------------	----	---------------	----	--------------------------------------	----	--	----	---------------	----	--------------	----	-------------	----	-------------	----	----------	----	----------	----	------------	----	--------------	----	---------------------	----	----------	----	------------	--

46 3.3.2 種菌之製備 47 3.3.3 基礎培養條件 47 3.3.4 分析方法 47 3.3.5 計算方法 49 3.4 實驗設計 53 3.4.1 環境因子之最適化及激博素生產菌之比較 54 3.4.1.1 菌絲密度 54 3.4.1.2 接菌量 54 3.4.1.3 發酵容積 55 3.4.1.4 振盪轉速 55 3.4.1.5 培養溫度 55 3.4.2 培養基組成之最適化 55 3.4.2.1 緩衝劑用量 56 3.4.2.2 碳源種類 56 3.4.2.3 碳源濃度 56 3.4.2.4 氮源種類 56 3.4.2.5 氮源濃度 57 3.4.3 直交矩陣設計 57 第四章 結果與討論 60 4.1 環境因子之影響 60 4.1.1 激博素發酵生產時程 60 4.1.2 菌絲密度之影響 63 4.1.3 接菌量之影響 71 4.1.4 發酵容積之影響 74 4.1.5 振盪轉速之影響 77 4.1.6 生長溫度之影響 80 4.1.7 較佳菌株之篩選 82 4.2 培養基組成之影響 85 4.2.1 緩衝劑之影響 85 4.2.2 碳源種類之影響 87 4.2.3 碳源濃度之影響 89 4.2.4 氮源種類之影響 91 4.2.5 氮源濃度之影響 93 4.3 各種最適因子之組合 95 4.4 直交矩陣實驗 98 第五章 結論 108 第六章 參考文獻 111

REFERENCES

1. Aksoz, N., Cihangir, N. and Aksoz, E. (1994) The determination of the possibility of fungal gibberellic acid production by using rotating biodisc reactor. *J. Biology.*, 18(1) : 1-7.
2. Ameha, M., Skirvin, R. M., Mitiku, G., Bullock, D. and Hofmann, P. (1998) In vitro tendril and flower development in cucumber (*Cucumis sativus*) may be regulated by gibberellins. *J. Hort. Sci. Biotechnol.*, 73(2) : 159-163.
3. Ashman P. J., Mackenzie A. and Bramley P. M. (1990) Characterization of ent-kaurene oxidase activity from *Gibberella fujikuroi*. *Biochim. et Biophys. Acta.*, 1036 : 151-157.
4. Avalos, J. and Cerdá-Olmedo, E. (1999) Nitrogen availability and production of bikaverin and gibberellins in *Gibberella fujikuroi*. *FEMS Microbiol. Lett.*, 173 : 389-393.
5. Avalos, J., Cerdá-Olmedo, E. and Sanchez-Fernandez, R. (1997) Inhibition of gibberellin biosynthesis by nitrate in *Gibberella fujikuroi*. *FEBS Lett.*, 413 : 35-39.
6. Bandelier, S., Renaud, R. and Durand, A. (1997) Production of gibberellic acid by fed-batch solid state fermentation in an aseptic pilot-scale reactor. *Process Biochem.*, 32(2) : 141-145.
7. Barendse, G. W. M., Werken, P. H., and Takahashi, N. (1980) High performance liquid chromatography of gibberellins. *J. Chromatogr.*, 198 : 449-455.
8. Bearder, J. R., Beale, M. H., Mac Millan, J. and Phinney, B. O. (1983) Diterpene acids from *Helianthus* species and their microbiology conversion by *G. fujikuroi* Mutant B1-41a. *Phytochem.*, 22(4) : 875-881.
9. Bearder, J. R., Beale, M. H., Mac Millan, J., Phinney, B. O., Hanson, J. R. and Willis, C. L. (1982) Gibberellin A13 7-aldehyde : A proposed intermediate in the fungal biosynthesis of gibberellin A3. *Phytochem.*, 21(9) : 2225-2230.
10. Borrow, A., Brian, P. W., Chester, V. E., Curtis, P.J., Hemming, H. J., Henehan, C., Jefferys, E. G., Lloyd, P. B., Nixon, I. S., Norris, G. L. F. and Radley, M. (1955) Gibberellic acid, a metabolic product of the fungus *Gibberella fujikuroi* : some observations on its production and isolation. *J. Sci. Food Agric.*, 6 : 340-342.
11. Borrow, A., Brown, S., Jefferys, E. G., Kessel, R. H. J., Lloyd, E. C., Rothwell, A., Rothwell, B. and Swait, J. C. (1964) Metabolism of *G. fujikuroi* in stirred culture. *Can. J. Microbiol.*, 10 : 407-409.
12. Borrow, A., Jefferys, E. G., Kessell, R. H. J., Lloyd, E. C., Lloyd, P. B. and Nixon, I. S. (1961) The metabolism of *Gibberella fujikuroi* in stirred culture. *Can. J. Microbiol.*, 7 : 227-230.
13. Brian, P. W., Elson, G. W., Hemming, H. G. and Radley, M. (1955) The plant growth promoting properties of gibberellic acid, a metabolic product of the fungus *Gibberella fujikuroi*. *J. Sci. Food Agric.*, 5 : 602-605.
14. Bruckner, B. and Blechschmidt, D. (1986) Die mikrobiologische synthese von gibberellinen. *J. Basic Microbiol.*, 26 : 483-484.
15. Bruckner, B. and Blechschmidt, D. (1991) The gibberellin fermentation. *CRC Crit. Rev. Biotechnol.*, 11(2) : 163-192.
16. Bruckner, B., Blechschmidt, D., Sembdner, G. and Schneider, G. (1989) Fungal gibberellin production, in biotechnology of vitamins, pigments and growth factor. *Vandamme.*, 21 : 1064-1069.
17. Bu ' Lock, J. D., Detry, R. W., Hostalek, Z. and Shakarchi, A. (1974) Regulation of secondary biosynthesis in *Gibberella fujikuroi*. *Trans. Br. Mycol. Soc.*, 62 : 377-378.
18. Chailakhyan, M. K. and Khryanin, V. N. (1978) The role of roots in sex expression in hemp plants. *Planta.*, 138 : 185-188.
19. Chapman, D. J. and Regan, M. A. (1980) Evolution of biochemical pathway: evidence from comparative biochemistry. *Ann. Rev. Plant Physiol.*, 31 : 639-642.
20. Chen, H. C. (1993) Citric acid production by *Aspergillus foetidus* in batch and fed-batch culture. *Food Biotechnol.*, 7(3) : 221-223.
21. Chen, H. C. and Liu, T. M. (1997) Inoculum effects on the production of -linolenic acid by the shake culture of *Cunninghamella echinulata* CCRC 31840. *Enz. Microb. Technol.*, 21 : 137-142.
22. Cross, B. E., Galt, R. H. B., Hanson, J. R., Curtis, P. J. and Grove, J. F. (1963) New metabolites of *Gibberella fujikuroi* II. *J. Chem. Soc.*, 6 : 2937-2943.
23. Crozier, A. (1981) Aspects of the metabolism and physiology of gibberellins. *Adv. Bot. Res.*, 9 : 134-149.
24. Cruz, P. M., Christen, P. and Farres, A. (1993) Medium optimization by a fractional factorial design for lipase production by *Rhizopus delemar*. *J. Ferment. Bioeng.*, 76 : 94-97.
25. Curtis, P. J. and Corss, B. E. (1954) Gibberellic acid. A new metabolite from the culture filtrates of *Gibberella fujikuroi*. *Chem. Ind.*, 1 : 1066-1072.
26. Darken, M. A., Jensen, A. L. and Shu, P. (1959) Production of gibberellic acid by fermentation. *Appl. Microbiol.*, 7 : 301-306.
27. De Conejos, R. F. and De Campo, G. G. (1975) Production de gibberellinas por fermentation de un medio contenido melaza de cana de azucar como fuente de carbono. *Arch. Bioquim. Quim. Farm.*, 20 : 39-46.
28. Diaz, C. E., Fraga, B. M., Gonzalez, A. G., Gonzalez, P., Hanson, J. R. and Hernandez, M. G. (1984) The microbiological transformation of some trachylobane diterpenoids by *Gibberella fujikuroi*. *Phytochem.*, 23(12) : 2813-2816.
29. Dockerill, B. and Hanson, J. R. (1978) The fate of C-20 in C19 gibberellin biosynthesis. *Phytochem.*, 17 : 701-704.
30. Domenech, C. E., Giordan, W., Avalos, J. and Cerdá-Olmedo E. (1996) Separate compartment for the production of sterols, carotenoids, and gibberellins in *Gibberella fujikuroi*. *Eur. J. Biochem.*, 239 : 720-725.
31. Dong, Y. H., Yao, J. L. and Morris, B. A. (1998) Identification of pollination-induced genes from the ovary of apple (*Malus domestica*). *Sexual Plant Reprod.*, 11(5) : 277-283.
32. Dorota, L. Z., Chelkowski, J., Foremska, E. and Piasecki, M. (1996) Biosynthesis of gibberellic acid (GA3) and mycotoxins by *F. moniliforme* sheldon and other species of *Liseola* section. *Natural toxins*, 4 : 228-233.
33. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956) Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, 28(3) : 350-356.
34. Erzinkyan, L. A. (1981) Gibberellin-growth stimulator for lactic acid bacteria. *Biol. Zh. Arm.*, 34 : 1188-1192.
35. Franck, D. M., W., Y., Ben Y. S. and Beachy, R.N.

(1998) In vitro stem elongation of sweet pepper in media containing 24-epi-brassinolide. *Plant Cell Tissue and Organ Culture*, 53(2): 79-84. 36. Fray, R. G., Wallace, A., Fraser, P. D., Hedden, P., Bramley, P. M. and Grierson, D. (1995) Constitutive expression of a fruit phytoene synthase gene in transgenic tomatoes causes dwarfism by redirecting metabolites from the gibberellin pathway. *The Plant J.*, 8(5) : 693-701. 37. Frederick, D. B., Edward, C. Y. and Richard, P. P. (1996) Far-red light stimulates internode elongation, cell division, cell elongation, and gibberellin levels in bean. *Can. J. Bot.*, 74 : 743-752. 38. Giordano W., Avalos J., Cerdá-Olmedo E. and Domenech E. (1999) Nitrogen availability and production of bikaverin and gibberellins in *Gibberella fujikuroi*. *FEMS Microbiol. Lett.*, 173 : 389-393. 39. Grindal, G., Junntila, O., Reid, J. B. and Joe, M. (1998) The response to gibberellin in *Pisum sativum* grown under alternating day and light temperature. *J. Plant Growth Regul.*, 17(3) : 161-167. 40. Holme, T. and Zacharias, B. (1985) Gibberellic acid formation in continuous culture. *Biotechnol. Bioeng.*, 7 : 405-410. 41. Hong, S. J. and Lee S. K. (1996) Effect of cytokinin and gibberellin on ripening of tomato fruit. *J. Kor. Soc. Hort. Sci.*, 37(3) : 374-379. 42. Hori, S. (1998) Some observations on "bakanae" disease of the rice plant. *Mem. Agric. Res. Sta. Tokyo*, 12 : 110-111. 43. Jacobsen, J. V. (1987) Regulation of protein biosynthesis in aleurone cells by gibberellin and abscisic acid. *Biochem. Physiol.*, 38 : 419-422. 44. Jefferys, E. G. (1970) The gibberellin fermentation. *Adv. Appl. Biol.*, 13 : 283-287. 45. Jones, R. L. (1987) The role of gibberellins in plant cell elongation. *CRC Crit. Rev. Plant Sci.*, 1 : 23-28. 46. Jose E., Nava S., Jean-Noel B. and Daniel T. (1989) Physiological and morphological modification in immobilized *Gibberella fujikuroi* mycelia. *Appl. Environ. Microbiol.*, 55(9) : 2377-2384. 47. Kahlon, S. S. and Malhorta, S. (1986) Production of gibberellic acid by fungal mycelium immobilized in sodium alginate. *Enz. Microbiol. Technol.*, 8 : 613-620. 48. Kashem, M. A., Itoh, K., Hayakawa, T., Hirai, N., Chigashi, H. and Mitsui, T. (1998) Effect of (+)-8', 8', 8' - trifluoroabscisic acid on alpha — amylase expression and sugar accumulation in rice cells. *Planta*, 205(3) : 319-326. 49. Kato, T. and Ito, H. (1962) Physiological studies on the promotive effect of gibberellic acid on the growth of the cereal plant. *Tohoku J. Agro. Res.*, 13 : 109-117. 50. Kawanabe, Y., Yamane, H., Murayama, T., Takahashi, N. and Teruko, N. (1983) Identification of gibberellin A3 in mycelia of *Neurospora crassa*. *Agric. Biol. Chem.*, 47 : 1-6. 51. Kennedy, M. J., Reader, S. L. and Davies, R. J. (1994) The kinetics of developing fermentation media. *Process Biochem.*, 29 : 529-534. 52. Kumar, P. K. R. and Lonsane, B. K. (1987) Gibberellic acid by solid state fermentation: consistent and improved yields. *Biotechnol. Bioeng.*, 30 : 267-272. 53. Kumar, P. K. R. and Lonsane, B. K. (1988) Immobilized growing cells of *Gibberella fujikuroi* P-3 for production of gibberellic acid and pigment in batch and semi-continuous cultures. *Appl. Microbiol. Biotechnol.*, 28 : 537-542. 54. Kumar, P. K. R., Sankar, K. U. and Lonsane, B. K. (1991) Purification of gibberellic acid from the concomitant products produced during solid state fermentation. *J. Chem. Eng.*, 46(2) : B53-B58. 55. Kurosawa, E. (1926) Experimental studies on the nature of the substance excreted by the bakanae fungus. *Trans. Nat. Hist. Soc. Formosa*, 16 : 213-214. 56. Lang, A. (1970) Gibberellins : Structure and metabolism. *Ann. Rev. Plant Physiol.*, 21 : 537-570. 57. Lenton, J. R., Applefold, N. E. J. and Croker, S. J. (1994) Gibberellins and -amylase gene expression in germinating wheat grains. *Plant Growth Regul.*, 15 : 261-270. 58. Lewer, P. and MacMillan, J. (1983) Effect of compactin on the incorporation of mevalonolactone into gibberellic acid by *Gibberella fujikuroi*. *Phytochem.*, 22(2) : 602-603. 59. Loreti, E., Guglielminetti, L., Yamaguchi, J., Gonzali, S., Alpi, A. and Perata, P. (1997) Effect of anoxia on gibberellic acid — induced protease and beta-amylase processing in barley seeds. *J. Plant Physiol.*, 152(1) : 44-50. 60. Lu, J., Lamikanra, O. and Leong, S. (1997) Induction of seedlessness in 'Triumph' muscadine grape (*Vitis rotundifolia* Michx) by applying gibberellic acid. *Hort.*, 32(1) : 89-90. 61. MacMillan, J. and Suter, P. J. (1958) The occurrence of gibberellin A1 in higher plants: isolation from the seed of runner bean (*Phaseolus multiflorus*). *Naturwissenschaften*, 45 : 46-47. 62. Maddox I. S. and Richert, S. h. (1977) Production of gibberellic acid using a dairy waste as the basal medium. *Appl. Environ. Microbiol.*, 22(1) : 201-202. 63. Matsukura, C., Itoh, S. I. and Nemoto, K. (1998) Promotion of leaf sheath growth by gibberellic acid in a dwarf mutant of rice. *Planta. (Berlin)*, 205(2) : 145-152. 64. Mayer, A. M. and Poljakoff-Mayber A. (1963) The germination of seeds. Pergamon Press. London. 65. McInnes, A. G., Smith, D. G., Durley, R. C., Pharis, R. P., Arsenault, G. P., Mac Millan, J., Gaskin, P. and Vining, L. C. (1977) Biosynthesis of gibberellins in *Gibberella fujikuroi*. *Can. J. Biochem.*, 55, 728. 66. Metz, B., Kossen, N. W. F. and Sujidam, J. C. (1979) The rheology of mould suspensions. *Biotechnol. Bioeng.*, 2 : 103-156. 67. Miller, W. R. and McDonald, R. E. (1994) Quality of preharvest GA3 — treated grapefruit after gamma irradiation and storage. *Proceed. Florida State Hort. Soc.*, 107 : 232-234. 68. Moss, M. O. and Smith, J. E. (1982) The biosynthesis of Fusarium mycotoxins, in the applied mycology of Fusarium. *Symp. Br. Mycol. Soc.*, 195 : 6-9. 69. Murado, M. A., Gonzalez, M. P., Pastrana, L., Siso, M. I. G., Miron, J. and Montemayor, M. I. (1993) Enhancement of the bioproduction potential of a amylaceous effluent. *Biores. Technol.*, 44 : 155-163. 70. Natalie, K. and Roni, A. (1998) Role of auxin and gibberellin in regenerative differentiation of tracheids in *Pinus* seedlings. *New Phytol.*, 138(3) : 461-468. 71. Nicoletta, R., Paola, M. and Vecchia-Francesca, D. (1998) Effects of seed chilling or GA3 supply on dormancy breaking and plantlet growth in *Cercis siliquastrum* L. *Plant Growth Regul.*, 25(1) : 53-61. 72. Ohana, O. and Wesis, D. (1998) Environmental and physiological factors regulate *Eryngium planum* flowering. *Israel J. Plant Sci.*, 46(1) : 47-51. 73. Palmer, G. H. (1971) The industrial use of gibberellic acid and its scientific basis — a review. *J. Inst. Brew.*, 80(1) : 13-30. 74. Pastrana, L. M., Gonzalez, Ma. P. and Murado, M. A. (1993) Production of gibberellic acid from mussel processing wastes in submerged culture. *Biores. Technol.*, 45 (3), 213-221. 75. Phinney, B. O. and Katsumi, M. (1982) Some generalization on the biosynthesis of gibberellins. *Chem. Regul.*, 6 : 44-49. 76. Plackett, R. L. and Burman, J. P. (1946) The design of optimal multifactorial experiments. *Biometrika*, 33 : 305-325. 77. Prema, P., Thakur, M. S., Prapulla, S. G., Ramakrishna, S. V. and Lonsane, B. K. (1983) Studies on recovery, purification and estimation of gibberellic acid produced by solid state fermentation. *Third Indian Covention of Food Scientists and Technologists India*, 17 : 88-89. 78. Prema, P., Thakur, M. S., Prapulla, S. G., Ramakrishna, S. V. and Lonsane, B. K. (1988) Gibberellin preparation using *Gibberella fujikuroi*, *Fusarium moniliforme* or *Botryodiplodia theobromae*. *India J. Microbiol.*, 28 : 78-80. 79. Priede, M. A., Vanags, J. J., Viesturs, V. E., Tucker, K. G., Bujalski, W. and Thomas, C. R. (1995) Hydrodynamic, physiological and

morphological characteristics of *Fusarium moniliforme* in geometrically dissimilar stirred bioreactors. *Biotechnol. Bioeng.*, 48 (3) : 266-277. 80. Qian, X. M., Du-Preez, J. C. and Killian, S. G. (1994) Factors affecting gibberellic acid production by *Fusarium moniliforme* in solid state cultivation on starch. *World J. Microbiol. Biotechnol.*, 10(1) : 93-99. 81. Rademacher, W. and Graebe, J. E. (1979) Gibberellin A4 produced by *Sphaceloma manihoticola*, the cause of the superelongation disease of cassava (*Manihot esculenta*). *Biochem. Biophys. Res. Commun.*, 91 : 35-39. 82. Rados, G. (1972) Biological methods for increasing the intensity of fermentation by yeasts. *Szeszipar*, 20 (1) : 9-20. 83. Rafael, F. M., Reyes, F., Peter, M. and Avalos, J. (1995) Gibberellin biosynthesis in gib Mutants of *Gibberella fujikuroi*. *J. Biol. Chem.*, 27 (25) : 14970-14974. 84. Rappaport, L. (1982) Applications of gibberellins in agriculture. *Plant Growth Sub.*, 6 : 12-18. 85. Righelato, R. C. (1979) The kinetics of mycelia growth in fungal walls and hyphal growthed. *J. Burr.* 4 : 385-401. 86. Salinas, Y. and Molina, J. (1996) Effect of gibberellic acid and of temperature on physiological quality of corn seeds. *Inform. Technol.*, 7 (2) : 43-39. 87. Sanchez-Marroquin, A. (1963) Microbiological production of gibberellic acid in glucose media. *Appl. Microbiol.*, 11 : 523-528. 88. Satvir, K., Anil, G. K. and Narinder, K. (1998) Gibberellin A3 reverses the effect of salt stress in chickpea (*Cicer arietinum* L.) seedlings by enhancing amylase activity and mobilization of starch in cotyledons. *Plant Growth Regul.*, 25(1) : 29-33. 89. Sawada, K. (1912) Disease of agricultural products in Japan. *Formosan Agric. Rev.*, 63 : 10-11. 90. Schuurink, R. C., Bakhuizen, R., Libbenga, K. R., Boulanger, F. and Sinjorgo, K. M. C. (1997) Dormant barley aleurone shows heterogeneity and a specific cytodifferentiation. *J. Cereal Sci.*, 25 : 27-36. 91. Sembdner, G., Gross, D., Liebisch, H. W. and Schneider, G. (1980) Biosynthesis and metabolism of plant hormones. *Encylopedia Plant Physiol.*, 9 : 281-300. 92. Sembdner, G., Weiland J., Aurich O. and Schrieber, K. (1968) Gibberellins in plant growth regulators. *Soc. Chem. Ind. Monograph.*, 31 : 70-72. 93. Shiao, M. S. (1983) Inhibition of gibberellin biosynthesis in *Gibberella fujikuroi* and germination of *Oryza sativa* by mevinolin. *Bot. Bull. Academia Sinica*, 9 : 135-143. 94. Shiozaki, S., Pan, M., Ogata, T., Horiuchi, S. and Kawase, K. (1998) Jasmonic acid effects on gibberellic acid — induced seedlessness in ‘ Neo Muscat ’ table grapes. *J. Hort. Sci. Biotechnol.*, 73(6) : 768-773. 95. Silveira, R. G., Kakizono, T., Takamoto, S., Nishio, N. and Nagai, S. (1991) Medium optimization by an orthogonal array design for the growth of *Methanosarcina barkeri*. *J. Ferment. Bioeng.*, 72, 20-25. 96. Southwick, S. M., Yeager, J. T. and Weis, K. G. (1997) Use of gibberellin on ‘ Patterson ’ apricot (*Prunus armeniaca*) to reduce hand thinning and improve fruit size and firmness : effects over three seasons. *J. Hort. Sci.*, 72(4) : 645-652. 97. Southwick, S. M., Yeager, J. T. and Zhou, H. (1995) Flowering and fruiting in ‘ Patterson ’ apricot (*Prunus armeniaca*) in response to postharvest application of gibberellic acid. *Sci. Hort.*, 60 : 267-277. 98. Stodola, F. H., Raper, K. B., Fennell, D. I., Conway, H. F., Johns, V. E., Langford, C. T. and Jackson, R. W. (1955) The microbial production of gibberellins A and X. *Arch. Biochem. Biophys.*, 54 : 240-248. 99. Taguchi, T. and Konishi, S. (1987) Orthogonal arrays and linear graphs. *J. Bacteriol.*, 179(2) : 430-438. 100. Takahashi, N., Kitamura, H., Kawarada, A., Seta, Y., Takai, M., Tamura, S. and Sumiki. (1955) Biochemical studies on bakanae fungus. XXXIV. Isolation of gibberellins and their properties. *Bull. Agric. Chem. Soc. Jpn.*, 19 : 267-269. 101. Takatori, F. H., Lorene, A. O. and Eink, F. W. (1959) Gibberellin sprays on celery. *California Agri.*, 13(7) : 3-4. 102. Thomas, D. (1989) Physiological and morphological modification in immobilized *Gibberella fujikuroi* mycelia. *Appl. Environ. Microbiol.*, 55(9) : 2377-2384. 103. Tomasini, A., Fajardo, C. and Barrios-Gonzalez J. (1997) Gibberellic acid production using different solid state fermentation system. *World J. Microbiol. Biotechnol.*, 13(2) : 203-206. 104. Valero, D., Martinez—Romero, D., Serrano, M. and Riquelme, F. (1998) Postharvest gibberellin and heat treatment effects on polyamines, abscisic acid and firmness in lemons. *J. Food Sci.*, 63(4) : 611-615. 105. Vass, R. C. and Jefferys, E. G. (1979) Gibberellic acid. *Econom. Microbiol.*, 3 : 421-422. 106. Wang, G., Zong, Y., Liang, L. and Wang, Y. (1998) Study on the storage technique for peaches : I. Effect of maturity and preharvest treatment on the storage performances. *Forest Research*, 11(1) : 30-33. 107. Wang, P. C. (1988) On the construction of some orthogonal main plans contrasts and factor combinations for two-level fractional factories designs of resolution III, IV, and V. *J. Qual. Technol.*, 20(4) : 267-272. 108. Wu, C. F. J. (1989) Construction of supersaturated designs through partially aliased interactions. *Biometrika*, 80 : 661-669. 109. West, C. A. and Phinney, B. O. (1956) Properties of gibberellin-like factors from extracts of higher plants. *Nature*, 178 : 1070-1074. 110. Woitek, S., Unkles, S. E. and James, R. K. (1997) 3-Hydroxy - 3 - methylglutaryl - CoA reductase gene of *Gibberella fujikuroi* : isolation and characterization. *J. Hort. Sci. Biotech.*, 72(4) : 108-112. 111. Yabuta, T. (1935) Biochemistry of the “ bakanae ” fungus of rice. *Agric. Hort.*, 10 : 17-25. 112. Yabuta, T., Kamebe, K. and Hayashi, T. (1934) Biochemistry of the “ bakanae ” fungus of the rice, I. fusaric acid, a new product of the bakanae fungus. *J. Agric. Chem. Soc. Jpn.*, 10 : 1059-1062. 113. Yabuta, T., Sumiki, Y. and Uno, S. (1939) Biochemical studies of bakanae fungus of the rice. IV. The cultural conditions for producing gibberellin or fusaric acid. *J. Agric. Chem. Soc. Jpn.*, 15 : 1209-1222. 114. Yomo, H. (1960) Amylase-activating enzymes. IV. Amylase-activating activity of gibberellins. *Hakko Kyokaishi*, 18: 600-601. 115. Yury, A. R. and Genrich, I. B. (1991) Nitrogen Regulation of gibberellin biosynthesis enzyme complex in *Fusarium moniliforme*. *J. Biotechnol.*, 21 : 219-228. 116. Zacarias, L., Talon, M., Cheikh, W. B., Lafueente, M. T. and Primo-Millo E. (1995) Abscisic acid increases in non- growing and paclobutrazol—treated fruits of seedless mandarins. *Physiol. Plant.*, 95 : 613-619. 117. Zeigler, R. S., Powell, L. E., and Thurston, H. D. (1980) Gibberellin A4 produced by *Sphaceloma manihoticola*, causal agent of cassava superelongation disease. *Phytopathol.*, 70 : 589-597. 118. 王承丕 (1990) L24 和 L48 的各類直交表。中國工業工程學刊 , 2(1):11-15. 119. 白賜清 (1996) 工業實驗設計法。中華民國品質管制學會 , 台北市。 120. 宇國勝、孫守恭 (1978) 賽養及光線對稻苗徒長病菌產孢之影響。植保會刊 , 20:141-150。 121. 余淑美 (1979) 不同寄主來源 *Fusarium moniliforme* 之生物學比較研究。國立中興大學植物病理研究所碩士論文。 122. 林金和 (1989) GA 生合成及其合成抑制之新近發展。科學發展月刊 , 17(2):142-147。 123. 林信山、張林仁 (1988) 植物生長調節劑在園藝作物之應用。台灣省台中區農業改良場第十五次研討會專集 , 5-10。 124. 徐美珠、黃涵 (1977) 激動素對台灣北部西洋芹菜生長與抽苔之影響。台大農學院研究報告 , 17(2):114-126。 125. 高景輝 (1982) 植物荷爾蒙。華香園出版社 , 92-169。 126. 高銘木 (1988) 植物荷爾蒙的應用與展望。今日經濟 , 255:79-85。 127. 張平順、黃天成 (1995) 激動素對苦瓜生長之

影響。嘉義農專學報，217-223。 128. 張嘉倫 (1994) 屠宰場廢棄豬血之利用。大葉工學院食品工程研究所碩士論文。 129. 許文章 (1993) 激勃素對西洋芹菜生長之影響。嘉義農專學報，33:1-10。 130. 陳日光 (1997) 二水準田口直交表最高解析度因子配置之研究。中華工學院工業工程與管理研究所碩士論文。 131. 陳健忠、張喜寧 (1996) 溫度及 GA3 對瓜葉菊生長與開花之影響。台大農學院研究報告，36(3):143-150。 132. 曾美倉 (1991) GA3 濃度及處理時間對木瓜種子發芽促進之影響。台大農學院研究報告，31(4):30-39。 133. 黃天成、張平順、侯清利 (1992) 激勃素對青蒜生長之影響。嘉義農專學報，30:1-8。 134. 黃涵 (1978) 植物生長素對大蒜生長及結球之影響。中國園藝，24(5-6):197-209。 135. 劉哲明 (1995) -次亞麻油酸之發酵量產研究。大葉工學院食品工程研究所碩士論文。 136. 蕭吉雄 (1988) 改變蔬菜生活習性 - 植物生長調節物質在蔬菜上的應用。興農雜誌，236 : 4-12。 137. 韓錦隆 (1993) 植物激素及其交互作用對胡瓜花性表現與營養生長之影響。宜蘭農工學報，6 : 56-59。 138. 簡道南、陳昭湘、羅芳容、郭政寬 (1991) 植物生長調節劑 - GA 之開發研究。台肥月刊，31(10) : 42-43。 139. 鐘美珠 (1986) 激勃素與植株矮化基因的關係。科學農業，34(3-4):87-90。