

CONIDIUM AND HYPHA MUTANTS OF
COCHLIOBOLUS MIYABEANUS (ITO & KURIBAYASHI)
DRECHSLER EX DASTUR¹

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Abstract

Isolate V-4 is a mutant of *Cochliobolus miyabeanus* (Ito et Kurib.) Drechsler arose spontaneously among the ascospore isolation cultures, the progeny of the cross of isolates 8GE and WK1A. This mutant developed a dwarf colony on cultural media, and hyphae rapidly transformed from normal smooth tube shaped structure into thick-walled, bead-chain like ones after conidia germinated. Conidia are small, straight or slightly curved. Isolate V-4-X, a mutant derived spontaneously from V-4, also developed a dwarf colony with small conidia and conidia formed secondary conidia, i. e., while the first formed conidia still attaching on conidiophores, germinated and germ-tubes immediately developed into conidiophores on which new conidia produced. Mutant isolate L-8 is a albino which developed a white colony and greyish white conidia. All three mutant isolates were cross-fertile with isolate WK1C and produced mature ascospores. The results of analyses of characters among single ascospore cultures revealed that abinosis was genetically controlled by a pair genes. However, the features of small conidia, formation of dwarf colony and secondary conidia, and transformation of smooth tube shaped hyphae into thick-walled, bead-chain ones were not regulated by nuclear genes but suggested that extrachromosomal factor(s) might be involved in regulating the above-mentioned mutated characters.

Key words: *Cochliobolus miyabeanus*; conidium; hypha mutants.

Introduction

A mutant isolate of *Cochliobolus miyabeanus* which developed a dwarf colony on culture media arose spontaneously among ascospore isolation cultures of the cross between isolates 8GE and WK1A. The frequency of this mutant among ascospore progeny is irregular, i. e., from none to 5 mutant isolates were observed in 200 ascospore isolation cultures. No same type mutant has been observed in

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other mating among Taiwan isolates of *C. miyabeanus* from either *Oryza sativa* or *Zizania latifolia*, and in the mating between isolates KU-13 and WK1C. Another mutant, isolate V-4-X, which originated spontaneously from mutant isolate V-4, possesses a characteristics of microcyclic conidiation, i.e., the conidium germinated and developed into a short conidiophore and subsequently formed a conidium while still attached to a conidiophore. The newly formed conidium was termed secondary conidium (Leonard, 1973). This feature of development seldom occurred in *C. miyabeanus*. The conidia of both mutants are obclavate, usually straight but rarely curved, and smaller than those of the wild type. An albino mutant isolate L-8 which developed a white colony on culture media, was isolated from a brown leaf spot of *Zizania latifolia* collected at Puli.

This report presented here includes the description of morphology of mutants mentioned above and the investigation on the inheritance of mutated characters, i.e., albinosis, small type conidium, bead-chain like hypha transformation and dwarf colony (slow mycelial growth).

Materials and Methods

Cochliobolus miyabeanus isolates. -Isolate WK1C, a normal type of *C. miyabeanus*, isolated from a rice brown leaf spot collected at Wuankuei, Yulan County is light-dependent for conidiophore formation. Conidia, 63-153 (109) × 14-22 (17) μ ; 6-14 septate. This isolate is cross-fertile with the following three mutants of *C. miyabeanus*: Isolate V-4, a mutant derived spontaneously among the progeny of cross between isolates 8GE and WK1A. This mutant developed a dwarf colony on V-8 juice agar, potato dextrose agar, corn meal agar, oat meal agar, and malt extract agar. Hyphae of this mutant rapidly transformed from smooth, septated tube-like structure into thick-walled, bead-chain ones (Fig. 1). Conidia obclavate, straight or slightly curved, 36-73 (51) × 15-22 (19) μ , and light-independent for conidiation. Isolate V-4-X, a mutant derived spontaneously from isolate V-4, also developed a dwarf colony, conidia obclavate, straight or slightly curved, 25-64 (43) × 17-22 (18) μ , 1-6-septate, conidia germinated and short germtubes immediately developed into conidiophores on which new conidia produced (Fig. 2), conidia produced light-independently. Isolate L-8, an albino mutant isolated from *Zizania latifolia* brown leaf spot collected at Puli, developed a white colony on V-4 juice agar and produced light greyish conidia with typical shape and size of *C. miyabeanus*.

Pseudothecium Formation. -Two compatible isolates, i.e., isolate WK1C and each of the three mutants mentioned above, were placed on opposite ends of a section of sterile corn leaf on Sach's medium (Nelson, 1957). Pseudothecia with mature ascospores formed three weeks after incubation at 23°C under 14 h photoperiod

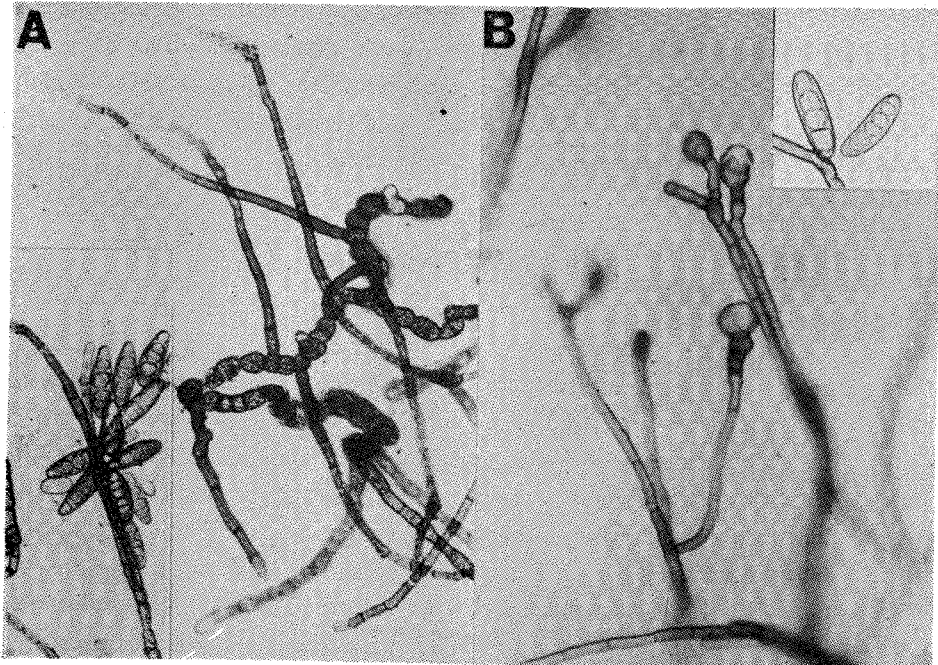


Fig. 1. A. Thick-walled, bead-chain like hyphae with small conidia of isolate V-4 of *Cochliobolus miyabeanus*; B. Conidiophores with swelling tips forming no conidia and those formed conidia of isolate X-4. (200 \times)

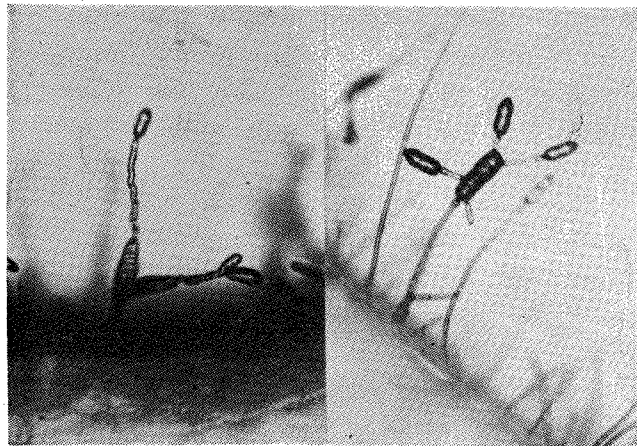


Fig. 2. Formation of secondary conidia of isolate V-4-X of *Cochliobolus miyabeanus*. (90 \times)

of cool white fluorescent light.

Distinctive characters such as conidium size, light-dependence for conidiation, and colony morphology were examined one week after incubation of ascospore isolation cultures at 25°C under continuous darkness. For those cultures which did not conidiate at darkness were subcultured and incubated at 25°C under 14 h illumination and 10 h dark regime to induce conidiation.

Results and Discussion

L-8 × *WK1C*

All the pseudothecia produced are dark black, indicating that isolate *L-8* has no capacity to form protothecia and acts only as male. Ueyama and Tsuda (1976) reported that an albino isolate A23 of *C. miyabeanus* possessed capacity to form protothecia and spermatia, and when it mated with a compatible natural isolate 3B both black and white pseudothecia were produced. However, another albino isolate UK-13W produced only black pseudothecia when mated with a compatible normal isolate A. The albino isolate *L-8* apparently behaved only as male like isolate UK-13W. Among 245 single ascospore isolation cultures 120 developed white colonies and 125 were normal, i.e., developed colonies with dark olivaceous brown. The ratio of two phenotypes approaches 1:1. This result clearly indicates that albinism is controlled by a pair allelic genes. Similar result was also demonstrated by Leonard (1975) on albino mutants of *C. carbonum*.

V-4-X × *WK1C*

Of total 659 single ascospore isolation cultures incubated at darkness, 263 were similar to parental isolate *WK1C* in cultural characters; 266 were same as isolate 8GE described in the previous report (Chang, 1975); 70 were with dwarf colonies, light independent for conidiation and with smaller conidia, colony morphology somewhat different from the parental type *V-4-X* with more conidia produced on the central area of colony, and produced secondary conidia; and 60 were light-dependent for conidiation, conidia smaller, and dwarf colony. The results demonstrated that light dependence for conidiation was genetically controlled by a pair of allelic genes; 336 of total ascospore cultures were light independent for conidiation and 323 were light dependent. Furthermore, among total single ascospore isolation cultures, 529 were with wild type conidia, normal mycelial growth and colony, while 130 were with smaller conidia and dwarf colony. Among progeny the ratio of wild type conidia to smaller type approaches 4 to 1. The fact showed that chromosomal inheritance could not explain the nature of inheritance of above mentioned characters. We suggested that extrachromosomal inheritance might be involved. Leonard (1973) studied the inheritance of a mutant of *C. carbonum* with

secondary conidium formation and obtained a very similar result as ours in segregation of wild type and type of forming secondary conidium among progeny of ascospore isolation cultures. He could not propose a definite explanation but suggested that extranuclear inheritance might play some role in the case he investigated. For further investigation on the phenomenon of segregation of conidial and cultural characters, a cross between isolates V-4-X-1 and WK1C was conducted. Isolate V-4-X-1, a progeny of the cross between isolates V-4-X and WK1C, is light independent for conidiation with smaller conidia and forming secondary conidium. The segregation of conidial and cultural characters among single ascospore isolation cultures from individual asci is shown in Table 1. The data, however, do not follow the ratio of Mendelian inheritance.

Table 1. Characters of progeny of the mating between isolate V-4-X and isolate WK1C

Ascus	Ascospores per ascus	No. of ascospore cultures grouped in			
		8GE type	WK1C type	V-4-X type	V-4-X-Ac type*
1	8	4	2	0	2
2	7	3	2	0	2
3	7	4	2	1	1
4	7	5	1	0	1
5	7	3	2	0	2
6	6	2	2	0	2
7	6	2	2	1	1

* Same as V-4-X in cultural character at dark condition but light dependent for conidiation.

V-4 × WK1C

Cultural characters of the ascospore progeny of the cross between these two isolates fell into 4 phenotypic groups: parental types, ie., V-4 and WK1C types, the third type is identical with isolate 8GE, and the last one is designated as X-4 which developed a compact small colony on the cultural media and produced no or few conidia (Fig. 1, B). Among 792 single ascospore isolation cultures the number of each group are: 8GE type, 311; WK1C type, 307; V-4 type, 79 and X-4 type, 95. The ratio of normal conidium type to small one is around 4:1. The results again only suggested that factor(s) regulate smaller conidium, slow mycelial growth and transformation of normal tube-shaped hyphae into thick-walled and bead-chain like structure might located in cytoplasm. Single ascospore isolation cultures classified as X-4 type produced conidia on sterilized corn leaf section placed on Sach's medium at 25°C under 14 h light and 10 h dark regime. The shape of conidium of type X-4 culture is similar to those produced by isolate V-4.

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胡麻葉枯病菌分生孢子及菌絲之變異株

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胡麻葉枯病菌 *Cochliobolus miyabeanus* (Ito et Kuribayashi) Drechsler ex Dastur, 無性世代 *Bipolaris oryzae* (Breda de Haan) Shoemaker 的變異株 V-4 是兩株胡麻葉枯病菌株 8GE 和 WK-1A 交配結果所得的子囊孢子子代中偶然所得的。其出現頻率並不規則，200 個子囊孢子單孢菌株中，有時一株也不出現，有時得一至五株不等。V-4 變異株的特徵是：分生膜孢子小 $36\sim73(51)\times15\sim22(19)\mu$ ，分生孢子發芽後通常慣有的管狀菌絲很快轉變成隔膜密集厚如唸珠狀之菌絲，貼生於培養基上或培養基內生長，生長緩慢，無氣生菌絲，和母株 8GE 一樣在無光照下也形成多量分生孢子。變異株 V-4-X 是由 V-4 自然衍生所得，其特徵與 V-4 同，分生孢子小，但具有形成次生分生孢子之性狀，即分生孢子尚未自分生孢子梗脫落即發芽，發芽管迅又轉化成分生孢子梗，其頂端再形成一分生孢子，白色菌株菌 L-8 是從埔里菱白上之病斑分離所得，以上三株變異菌株與菌株 WK1C 配對均能產生成熟之擬子囊殼及子囊孢子。單孢子囊孢子分離培養，性狀分析得知白化 (albino) 是一對核因子控制。因子囊孢子子代菌株中約一半為白化株，一半為正常株，而且子囊殼均為黑褐色，故推知 L-8 菌株只有雄性作用。分生孢子小型，次生分生孢子之形成以及菌絲轉化成節狀唸珠狀之性狀，似非核因子所控制，因其出現性狀並不遵循孟德爾之比率。