

THE PHENOLIC COMPOUNDS DIFFERENTIATION BETWEEN THE CROSS OF *ORYZA SATIVA* (AA) AND *O. AUSTRALIENSIS* (EE)¹

YUAN KU, LIN WU and H. W. LI²

(Received Feb. 7, 1969)

Introduction

In recent years, with the technique of paper chromatography, the patterns obtained from phenolic compounds were generally used as a reliable indicator on taxonomic or evolutionary investigation (Turner 1959, Alston 1962, Levin 1966). In our laboratory, we also used the biochemical method to differentiate the phenolic compounds in *Oryza* (Wu *et al.* 1967) and proved that the distributions of these compounds were closely related to the ancestral relationship. It would indicate that the phenolic profiles seemed to fall under the biochemical control of gene of genes.

In the past few years, we have isolated a series of alien additional lines from currently backcrosses between *Oryza sativa* (Taichung 65) and *O. australiensis*. An extra chromosome from *O. australiensis* was added to the plant with *O. sativa* background (Simplex) (Wu *et al.* 1967). In this experiment, using the alien additional lines, we intended to understand whether the chromatographic patterns could provide a dependable indicator of the alien chromosomes.

Materials and Methods

The materials used for this study were collected from the leaf blades of the parent plants (*Oryza sativa* and *O. australiensis*), F₁ hybrid, triploid, and 12 alien additional lines.

In order to extract the components more complete, the leaf tissues were not extracted with reflexed method as in Wu's experiment (1967). 0.5 gram powered leaves (selected for uniform condition) were extracted for 24 hr in the dark in 1% HCl in 80% methanol. The volume of extracting fluid should barely cover the leaf tissue (Torres 1964). Extracts were developed in two dimensions by the ascending method (Wu *et al.* 1967).

All of these dried chromatograms were examined (1) in ultraviolet light

¹ Paper No. 81 of the Scientific Journal Series, Institute of Botany, Academia Sinica.

² Research Assistants and Research Fellow, respectively.

alone (2) in day-light with the presence of ammonia vapor (3) in ultraviolet light with the presence of ammonia vapor.

The whole process was repeated more than four times.

Results and Conclusion

Genomic Designation:

AA=*O. sativa*

EE=*O. australiensis*

AE= F_1 Hybrid

AAE=Triploid

AA+E_x=Simplex

From two-dimensional studies, there were some variations present but most consistent appearing spots are represented in the diagrams Plates I and II. A survey of the chromatographic patterns of AA and EE revealed a striking difference in their phenolic profiles. And those in the derivatives were more

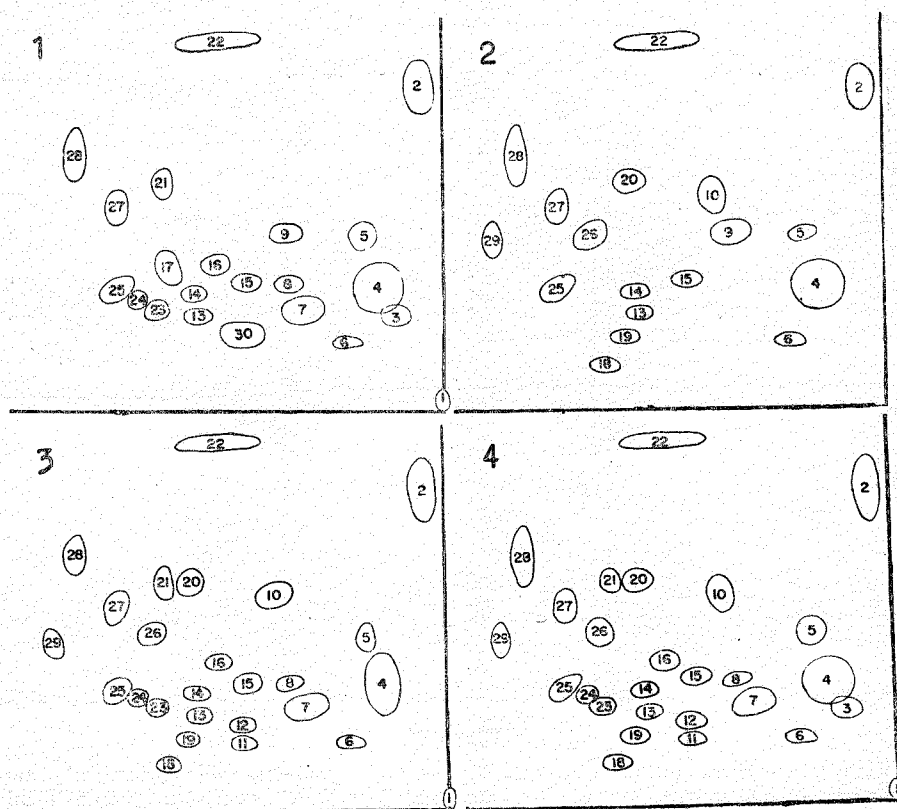


Plate I.

Two dimensional chromatographic patterns of phenolic compounds of leaf extracts. The spots were detected under ultraviolet light with the presence of ammonia vapor.

Fig. 1. *O. sativa* Fig. 2. *O. australiensis* Fig. 3. F_1 Hybrid Fig. 4. Triploid

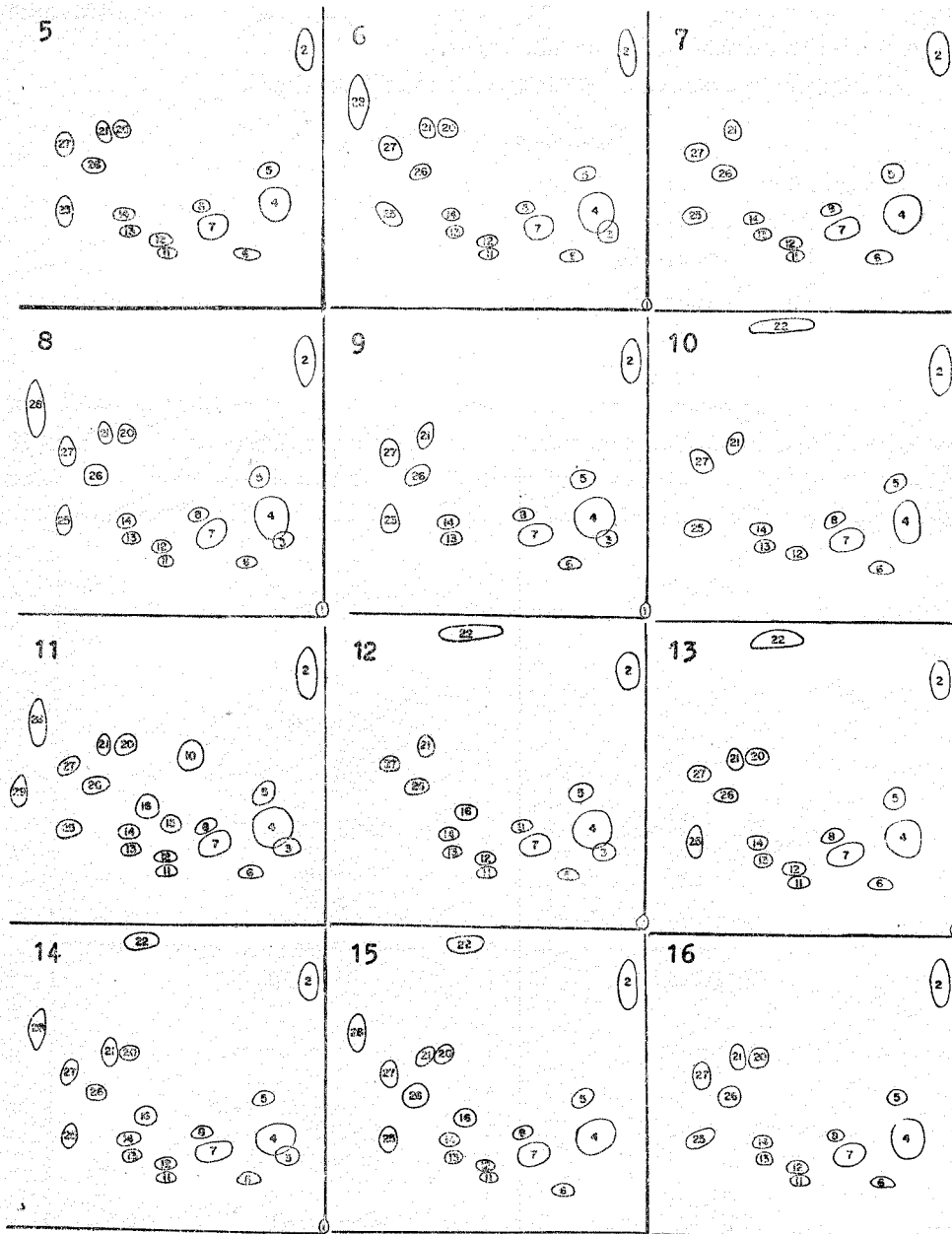


Plate II.

Composite representation of the two-dimensional chromatographic patterns of phenolic compounds of leaf extracts of 12 alien additional lines which were isolated from Triploid (AAE) × *O. sativa* (AA). The spots were detected under violet light with the presence of ammonia vapor.

Fig. 5. Simplex AA+E₁

Fig. 8. Simplex AA+E₄

Fig. 11. Simplex AA+E₇

Fig. 14. Simplex AA+E₁₀

Fig. 6. Simplex AA+E₂

Fig. 9. Simplex AA+E₅

Fig. 12. Simplex AA+E₈

Fig. 15. Simplex AA+E₁₁

Fig. 7. Simplex AA+E₃

Fig. 10. Simplex AA+E₆

Fig. 13. Simplex AA+E₉

Fig. 16. Simplex AA+E₁₂

or less different due to their different genomic constitution. The results are summarized in Table 1. All of the spots as appeared in each sample as they appeared in all the plate figures whose Rf values and colors in different light with presence or absence of ammonia vapor are shown in Table 2.

Table 2. Spots of phenolic compounds extracted from the leaves of *O. sativa* (AA), *O. australiensis* (EE), F₁ Hybrid (AE), Triploid (AAE), and 12 alien additional lines (AA+E_x) detected under different treatments

Spot No.	Rf value		Color*		
	First dimension	Second dimension	Day-light+NH ₃	UV light	UV light+NH ₃
1	0.02	0.03	—	W	W
2	0.81	0.06	LG	—	YG
3	0.27	0.11	—	W	WY
4	0.34	0.14	YG	Br	Y
5	0.44	0.16	—	—	YG
6	0.17	0.22	—	—	WO
7	0.27	0.33	—	—	O
8	0.33	0.37	—	—	W
9	0.45	0.36	YG	Br	G
10	0.53	0.40	—	—	O
11	0.18	0.48	LG	Br	DG
12	0.23	0.48	LG	Br	DG
13	0.25	0.58	LG	Br	DG
14	0.31	0.60	LG	Br	DG
15	0.34	0.47	—	—	Pi
16	0.39	0.54	—	—	Pi
17	0.38	0.64	LG	Br	G
18	0.12	0.65	—	—	Pi
19	0.17	0.61	—	—	Pi
20	0.60	0.58	—	Pi	Pa
21	0.60	0.66	—	W	WB
22	0.93	0.73	—	—	BG
23	0.29	0.69	LG	Br	DG
24	0.31	0.73	LG	—	G
25	0.32	0.77	—	B	PB
26	0.47	0.68	—	—	BG
27	0.54	0.77	—	B	PB
28	0.66	0.88	—	—	BG
29	0.43	0.90	—	—	BG
30	0.20	0.49	G	Br	G

* W=white Y=yellow G=green DG=dark green PB=purplish blue Pa=pale Br=brown Pi=pink LG=light green B=blue O=orange WO=white orange BG=blue green

From Table 2 it can be seen spots 10, 29 are responsible for the presence of E₇. Except these, it can be concluded only the genes responsible for the synthesis of different phenolic compounds are rather complicated. Making these identification of the chromosomes are almost an impossible job.

用濾紙分離法對栽培稻，澳洲野生稻及其十二種外加染色體系統 (alien additional lines) 之研究

顧 韜 吳 麟 李 先 聞

經過一連串的回交工作，本所已成功的將澳洲野生稻的染色體逐個分離出來，添加入栽培稻臺中 65 號中。由於不同外加染色體的影響，使原來臺中 65 號的性狀，都發生相當明顯的差異。因此從形態上，我們已可將 12 種外加染色體系統 (alien additional lines) 分辨出來。

近年來，濾紙分離法已廣泛地被應用在分類及種源的探測工作上。本實驗是嘗試用這種生化的方法，比較化合物在十二種系統中的異同及其與親本的相關性，以求作為另一種區別各個外加染色體的依據。

但由結果看來，這些芳香族化合物的合成，絕大多數都是多因子遺傳，而非由單條染色體控制，因此，用濾紙分離法，對芳香族化合物之分析，並不能應用於染色體之鑑定。

Literature Cited

- ALSTON, R. E. and B. L. TURNER. New techniques analysis of complex natural hybridization. Proc. Natl. Acad. Sci. **48**: 130-137, 1962.
- LEVIN, D. A. Chromatographic evidence of hybridization and evolution in *Phlox machlata*. Amer. Jour. Bot. **53**(3): 238-245, 1966.
- TURNER, B. L. and PALPH ALSTON. Segregation and recombination of chemical constitution in a hybrid swarm of *Baptisia laevicanlis* × *B. viridis* and their taxonomic implications. Amer. Jour. Bo. **46**: 678-686, 1959.
- TURNER, B. L. and D. A. LEVIN. A chromatographic study of *Cespitose zinias*. Amer. Jour. Bot. **51**: 639-643, 1964.
- WU, L., ORCHID M. Y. CHU and H. W. LI. A paper chromatographic study of phenolic compounds in *Oryza*. Bot. Bull. Aca. Sci. **8**: 91-101, 1967.
- WU, L., K. S. TSAI and H. W. LI. Cytogenetical studies of *Oryza sativa* L. and its related species. Bot. Bull. Aca. Sci. **8**: 165-170, 1967.