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Infection of Peanuts by *Puccinia arachidis*[1)]

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摘　　要

林益昇、陳際松、鄭瑤華 (1983)　***Puccinia arachidis*** **對花生侵染過程之研究　植保會刊 26: 3～10.（臺灣省農業試驗所植物病理系）**

本文以組織透化的方法研究 *Puccinia arachidis* 對不同花生品種（系）之侵染過程。品系 P. I. 315608 的葉片組織對侵入之病原菌呈過敏性反應，表現强抗病性。而品系 P. I. 314817 及 P. I. 259747 之抵抗性却促使已侵入，並已建立在寄主葉片組織之病原菌感染菌絲形成小而且少的夏胞子堆。又品系 P. I. 259747 之葉面，能使病原菌之夏胞子發芽率及附著器形成率較品種 TN-4 遲緩 3 個小時，這個現象或許在田間露水期間不够長的狀況下，可使這個品系因而逃避部份的感染源。

（關鍵字：花生銹病，*Puccinia arachidis*，侵染過程，抵抗性）

ABSTRACT

The infection processes of four accessions/varieties of peanut by *Puccinia arachidis* were studied in detail in unsectioned leaf tissue. Accession P. I. 315608 showed a hypersensitive reaction in leaf tissue to the pathogen to prevent its successful colonization. Accessions P.I. 314817 and P. I. 259747 were able to reduce the number and size of uredia in the infected leaves, when compared with susceptible variety TN-4. Moreover, a 3 hr of delay of spore germination and appressorium formation of *P. arachidis* on accession P. I. 259747 might be significant in reducing infection in the field, when the dew period was not long enough for the infection.

(Key words: peanut rust, *Puccinia arachidis*, infection processes, resistance).

INTRODUCTION

Three peanut accessions, P. I. 314817 (DHT-200), P. I. 259747 (Tarapoto) and P. I. 315608 (Israel Line 136) have been reported resistant to rust in most peanut grown areas in the world[3,6,12]. However, the mechanisms of resistance of these

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accessions are still controversial[3,4,6,10]. Lin[9] reported in 1981 that the resistance expression in terms of pustule numbers might be varied due to different inoculation methods. He pointed out that resistance could be overcome by a heavy load of inoculum. However, the resistant accessions had fewer, smaller pustules with less spores when compared with the susceptible variety TN-4. In these resistant hosts, the plant and pathogen are in a conflict. The plant may oppose the growth of *Puccinia arachidis* Speg. at any stage of the disease cycle including spore germination, appressorium formation, penetration, substomatal vesicle, infection hypha, haustorium and uredium development, and sporulation. We, therefore, investigated the infection processes of *P. arachidis* on peanut leaves of different accessions/varieties and hoped to determine the factors that reduced infections.

MATERIALS AND METHODS

Uredospores of *P. arachidis* were collected from 6-day-old pustules on variety TN-4 in the greenhouse as inoculum. Four peanut accessions/varieties were investigated: TN-4 (highly susceptible), P. I. 314817, P. I. 259747 (slow rusting) and P. I. 315608 (highly resistant). Peanut plants were grown in the greenhouse for 6 wk. Leaflets of 7 or 14 days old were used in the inoculation tests.

The abaxial surfaces of peanut leaflets were sprayed as evenly as possible with freshly harvested uredospore suspension (1 g/1000 ml) containing 0.1% wetting agent (Tween 80). The inoculated plants were then incubated at 28°C under a 12 hr photoperiod (1250 lux). Leaflets of 7 or 14 days old were detached at 3 hr after inoculation and then every 3 hr there after until 24 hr. The detached inoculated leaflets were sprayed with lactophenol cotton blue. Spore germination and appressorium formation of 500 spores were counted directly under a microscope for each test accession/variety.

Leaflets of another set of inoculated plants were detached at 9, 12, 15, 18, 21, 24, 36 or 504 hr after inoculation and treated by McBredy's staining method[10]. The substomatal vesicles of the fungus were counted to determine the infection. The development of infection hyphae and uredia were also observed to determine the extent of colonization. Incubation periods and pustule sizes were also recorded.

RESULTS

The number of stomata on abaxial surfaces of accession P. I. 314817 was more than that of other accessions/varieties (Table 1).

Table 1. The number of stomata/mm² on the abaxial surface of peanut leaves of different accessions

Accession	TN-4	P. I. 314817	P. I. 259747	P. I. 315608
No. of stomatum per mm²	100a[1)]	149 b	114 a	110 a

1) Values with same letter are not significantly different at 0.05 level by Duncan's Multiple Range Test.

Fresh uredospores in pustules of 6 days old had the highest germination percentage (more than 80%) on abaxial surfaces of peanut leaves of variety TN-4 (Fig. 1). Therefore, uredospores of this age were constantly used as inoculum in this study.

Uredospore germinated by the formation of an unbranched germ tube within 3 hr after inoculation. The germ tube

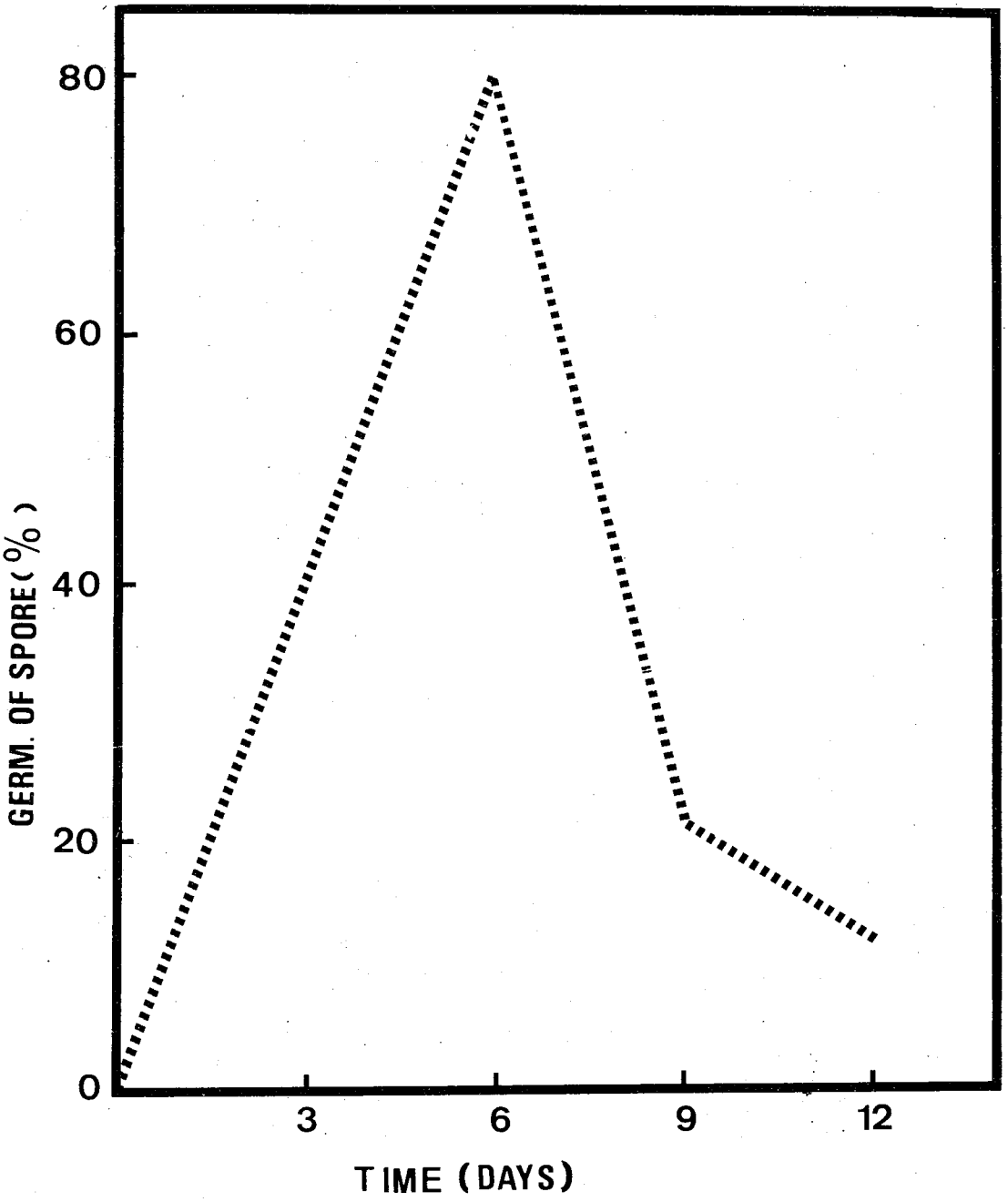


Fig. 1. Germination percentage of uredospores collected from fresh uredia at different times after their formation.

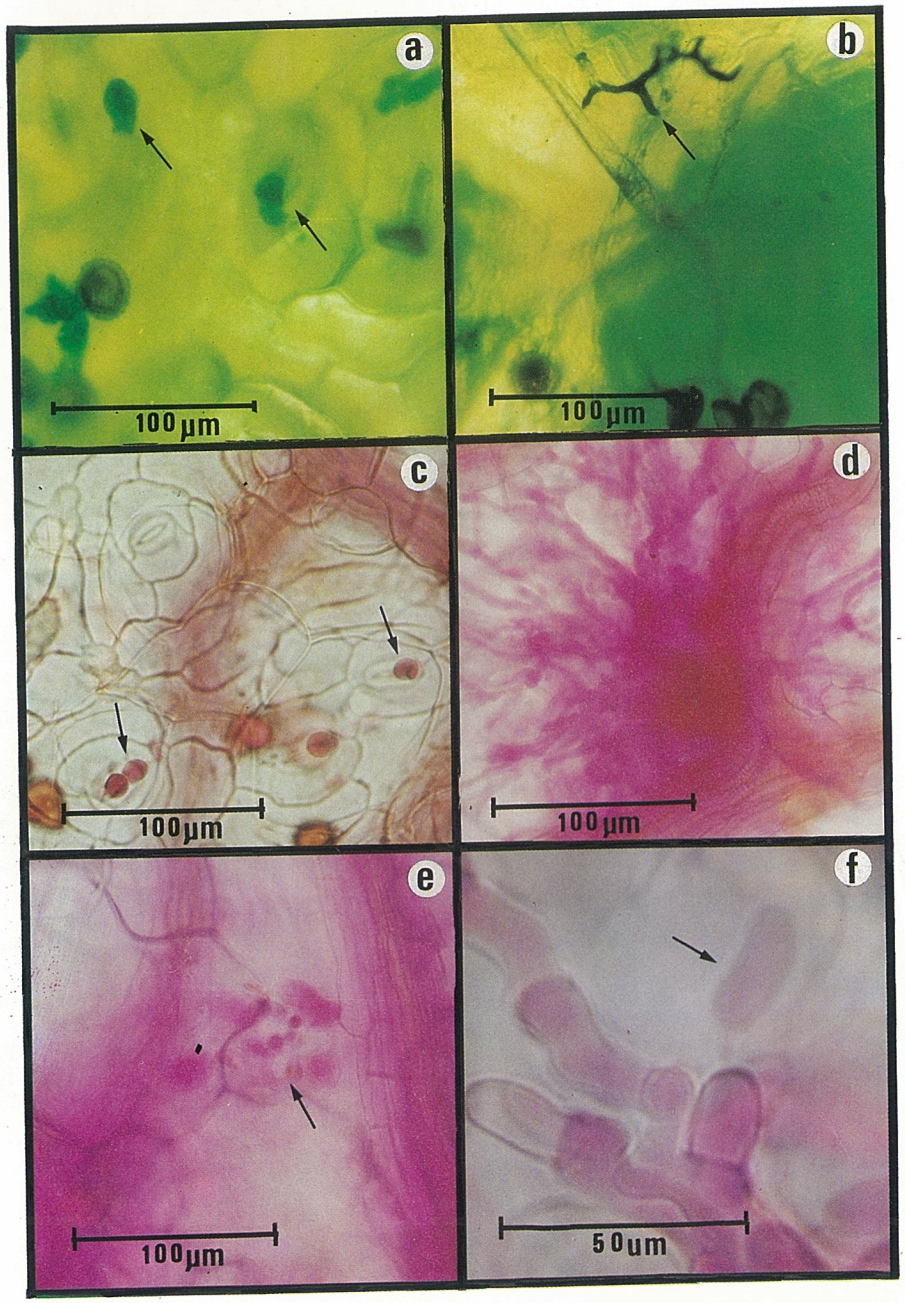


Fig. 2. Infection processes of *Puccinia arachidis* on abaxial surface of peanuts: a—appressoria (arrows) on or not on stomata, b—abnormal branched appressorium (arrow), c—one or two substomatal vesicles (arrows) in substomatal chamber, d—successful colonization with infection hyphae in leaf tissue of accessions/varieties TN-4, P. I. 259747, and P. I. 314817, e—failure of colonization with lysed infection hyphae (arrow) in leaf tissue of accession P.I. 315608, f—sausage shaped haustorium (arrow) in mesophyll cell.

directly contacted the first stoma encountered or passed one or more stomata before forming an appressorium. Appressoria formed within 9 hr by swelling the tip of a germ tube (Fig. 2a). Most of them were about the same size as the germ tube, but occassionally, some of them branched indefinately (Fig. 2b). The branched appressoria did not germinate. Percentages of uredospore germination and appressorium formation come to the peaks at 12 hr or 15 hr after inoculation for accessions/varieties P. I. 314817, P. I. 315608 and TN-4, and 15 or 18 hr for accession P. I. 259747, respectively (Figs. 3, 4).

A narrow infection peg would grow from an appressorium through the stomatal aperture and form a vesicle which could be stained clearly in the substomatal chamber by McBredy's method[10]. Frequently, one stoma might receive two penetrations and two vesicles could be observed in one substomatal chamber (Fig. 3e). The numbers of vesicles are more in variety TN-4, less in accessions P. I. 314817 and and P. I. 259747 and few in accession P. I. 315608 (Table 2). Several infection hyphae usually grew from the substomatal vesicle within 21 hr (Fig. 2d). However, the infection hyphae lysed in accession P. I. 315608 (Fig. 2e) From these infection hyphae, sausage shaped haustoria developed within adjacent mesophyll cells (Fig. 2f). Uredial development on all accessions/varieties was completed within 504 hr after inoculation. The incubation period had not much difference in the different test accessions/varieties (Table 3). The number and size of pustules were significantly different in all test accessions/varieties. They were more and larger in TN-4, less and smaller in P. I. 314817 and P. I. 259747 and none in P. I. 315608 (Table 2).

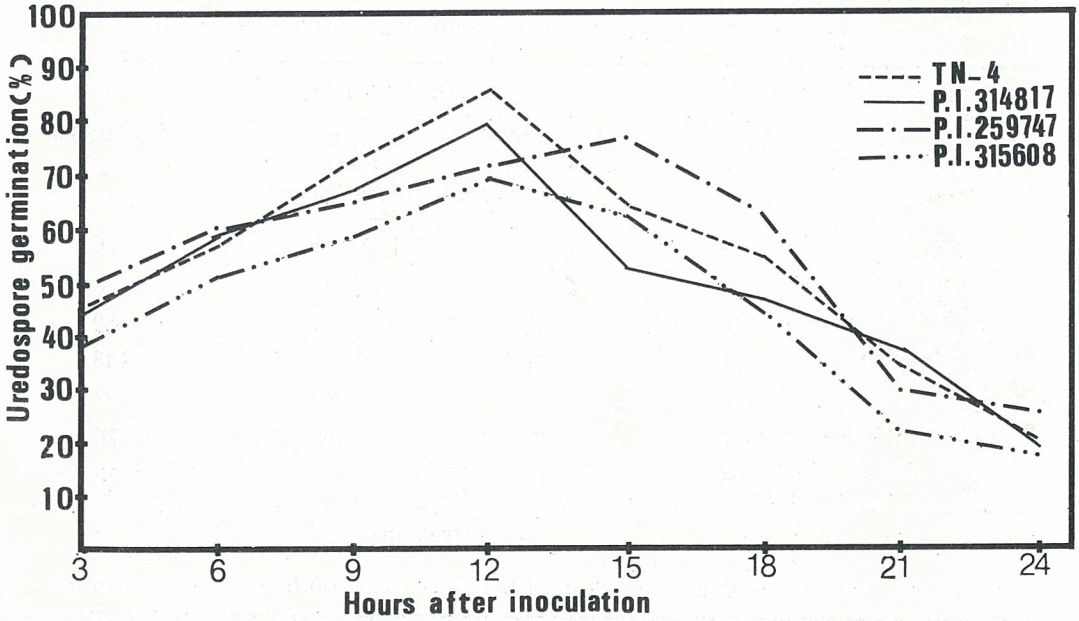


Fig. 3. Percentage of uredospore germination of *Puccinia arachidis* on the abaxial surface of peanut accessins/variety at different times after inoculation.

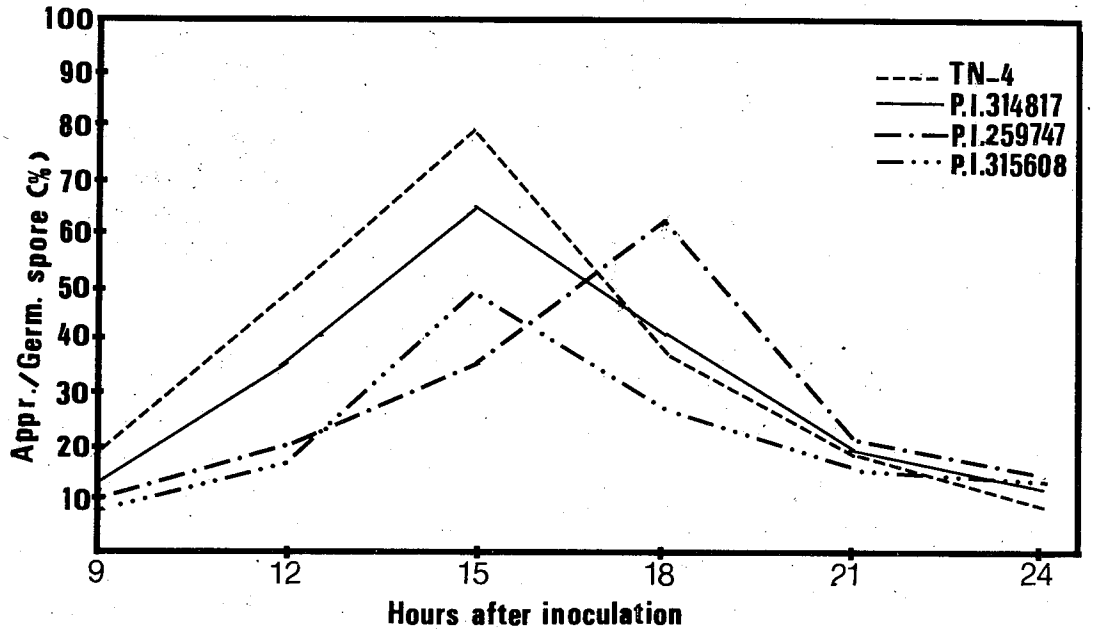


Fig. 4. Percentage of appressorium formation among the germinated uredospores of *Puccinia arachidis* on the abaxial surface of peanut accessions/variety at different times after inoculation.

Table 2. Number of substomatal vesicles and uredia of *Puccinia arachidis* in leaf tissue of peanut at different hours after inoculation

Time after inoculation (hr)	Accession/variety			
	TN-4	P. I. 314817	P. I. 259747	P. I. 315608
	Substomatal vesicle/50 mm²			
9	18	6	5	0
12	54	34	34	12
15	100	76	109	80
18	171	163	117	148
21	188	166	153	87
24	191	175	168	26
36	196	181	174	13
	Uredium[1)]			
504	32 a	24 b	20 b	0 c

1) Values with same letter are not significantly different at 0.05 level by Duncan's Multiple Range Test.

Table 3. The incubation period and pustule size of *Puccinia arachidis* on different accessions/varieties of peanut

Accession	Incubation Period (days)	Pustule size[1] (um × um)
TN-4	8	482 (193-593) × 461 (187-578)
P. I. 314817	9	273 (185-296) × 201 (169-210)
P. I. 259747	9	261 (184-269) × 232 (165-250)

1) Pustule sizes were measured at 21 days after inoculation.

DISCUSSIONS

Kono[3] stated that wax played little role but stoma number had correlation with the spore germination and appressorium formation of *P. arachidis* on peanut leaf surfaces. However, our results (Table 1) showed that the stoma number had no effect on infection. Cook[5] reported that stomata did not have to be opened to allow successful penetration and resistance of some cultivars, including P. I. 259747, to infection by *P. arachidis* appeared to be related to changing wetting resistance of the leaves when getting old. In other words, the abaxial surfaces of older leaves were less wettable and susceptible than those of younger ones. However, in the field, the disease progresses from older leaves up to younger leaves[9]. Therefore, the wetting resistance of the leaves may not be important in resistance expression. In our study, those factors were excluded by using 7 and 14-day-old leaves and a wetting agent (Tween 80) in the inoculation tests. The results (Figs. 3, 4) indicated that other factor(s), for example, sugar and amino acids exuded from leaf surface[8] might also be involved in the reduction of appressorium formation. The delay of 3 hr of spore germination and appressorium formation in P. I. 259747 may be significant in the field for reduced infections when the dew period is not long enough for the appressorium formation in this variety.

It was no difficulty for penetration of infection pegs to the substomatal chamber and the formation of vesicles in all test accessions/varieties. However, appressorium formation and vesicle formation were less in P. I. 315608 (Table 2). In this accession, short infection hyphae grew out from vesicles but lysed finially (Fig. 2e). The hypersensitive reaction stopped successful colonization, and this accession expressed a high resistance. Vesicle formation in accessions P.I. 314817 and P. I. 259747 were almost as high as that in variety TN-4, although the formation time was a little bit delayed (Table 2). These had good growth of infection hyphae, with sausage shaped haustoria (Fig. 2f), resulting in successful colonization, but the numbers of uredia formed in an unit area were less than that in variety TN-4. Cook[5] also reported that chloronemic flecks to become uredia were prevented at this stage in P. I. 259747. However, pustule number needs not to be closely correlated with vesicle number since hyphal growth may be aborted[7] or hyphae may fuse with each other and form more vigorous

structures[1] before pustule formation. Lin[9] indicated that less initial infection in P. I. 314817 and P. I. 259747 compared with variety TN-4 might be somewhat obscured late in the season if they are continually exposed to inoculum from susceptible plants in fields or exposed to a high concentrations of inoculum in greenhouse inoculation tests. Therefore, the smaller pustule size (Table 3) and less spore production[9] might account for the slow rusting of P. I. 314817 and P. I. 259747 in the field.

REFERENCES CITED

1. Akutsu, K., K. Ko and T. Misato. 1981. Role of conidial fusion in infection by *Botrytis cinerea* on cucumber leaves. Ann. Phytopath. Soc. Japan 47:15-23.
2. Bromfield, K. R. 1971. Peanut rust:A review of literature. J. Amer, Peanut Res. Edu. Assoc. 3:111-121.
3. Bromfield, K. R. and S. I. Cevario. 1970. Greenhouse screening of peanut (*Arachis hypogagae* L.) for resistance to peanut rust (*Puccinia arachidis* Speg.). Pl. Dis. Reptr. 54:381-383.
4. Cook, M. 1972. Screening of peanut for resistance to peanut rust in the greenhouse and field. Pl. Dis. Reptr. 56:382-386.
5. Cook, M. 1980. Host-parasite relationships in uredial infections of peanut by *Puccinia arachidis*. Phytopathology 70:822-826.
6. Hammons, R. O. 1977. Groundnut rust in the United State and the Caribbean. PANS 23:300-304.
7. Hooker, A.L. 1967. The genetics and expression of resistance in plants to rusts of the genus *Puccinia*. Ann. Rev. of Phytopathology 5:163-182.
8. Kono, A. 1977. On the uredospore germination of peanut leaf rust (*Puccinia arachidis* Speg.) on peanut leaves. Bulletin of the Faculty of Agriculture. Miyazaki Univ. 24(2): 225-234.
9. Lin, Y. S. 1981. Effects of inoculum concentrations on the resistance of peanuts to *Puccinia arachidis* Speg. J. of Agric. Res. of China 30:324-331.
10. McBredy, Mary C. 1936. A method of demonstrating rust hyphae and haustoria in unsectioned leaf tissue. Amer. J. of Botany 2:686-688.
11. McVey, D. V. 1965. Inoculation and development of rust on peanut grown in greenhouse. Pl. Dis. Reptr. 49:191-192.
12. Subrahmanyam, P., R. W. Gibbons, S. N. Nigam and V. R. Rao. 1980. Screening methods and further source of resistance to peanut rust. Peanut Science 7:10-12.