International Journal of Bio-resource and Stress Management https://pphouse.org/ijbsm.php

Crossref

IJBSM September 2022, 13(9):954-960 Print ISSN 0976-3988 Online ISSN 0976-4038 **Article** AR3045

Research Article *Stress Management*

DOI: HTTPS://DOI.ORG/10.23910/1.2022.3045

Early Interactions of Rust Pathogen *Puccinia arachidis* (Speg.) with Groundnut Genotypes Varying in Resistance

V. Ramya[1], S. A. Thilak[1], G. Uma Devi[1], Pushpavalli S. N. C. V. L.[2] and Hari K. Sudini[3]

[1]Dept. of Plant Pathology, [2]Dept. of Molecular Biology and Biotechnology, College of Agriculture, Rajendranagar, Professor Jayashankar Telangana State Agricultural University, Hyderabad, Telangana (500 030), India

[3]Dept. of Groundnut Pathology, Research Program-Asia, International Crop Research Institute for Semi-Arid Tropics, Patancheru, Hyderabad, Telangana (502 324), India

Open Access

Corresponding ramya.vittal@gmail.com

0000-0002-2692-5330

ABSTRACT

The study was conducted in the Department of Plant Pathology, College of Agriculture, Rajendranagar, Professor Jayashankar Telangana State Agricultural University, Hyderabad, India to understand the histopathological mechanisms of initial interaction of *Puccinia arachidis* with six groundnut genotypes. Fully expanded quadrifoliate leaves were inoculated with *P. arachidis* using the detached leaf assay. Five-mm leaf discs were cut at 6 hours after inoculation (hai) and each day until 5 days after inoculation (dai) and examined for histopathological interactions. Germination of urediniospores was detected during 6–8 hai and continued until 24 hai. There were no differences in pre-penetration and penetration among all the six genotypes. Differences in post-penetration were observed among the genotypes during 3–5 dai. In genotypes TMV 2 and K 6, extensive hyphal colonization was observed in the intercellular spaces of mesophyll cells by 4–5 dai indicating compatible interaction and susceptibility to rust disease. Sparse hyphal growth and corresponding mesophyll cell death at 3–5 dai in genotypes ICGV 171015 and ICGV 13229 indicated defense response by the host and moderate resistance. In genotypes ICG 11426 and GPBD 4, complete arrest of hyphal growth by 4–5 dai due to extensive mesophyll necrosis suggested incompatible interaction and resistance to rust. This is the first documentation of the histopathological description of the initial infection strategies of *P. arachidis* in the selected groundnut genotypes.

KEYWORDS: Disease resistance, groundnut rust, histopathology, infection, *Puccinia arachidis*

***Citation* (VANCOUVER):** Ramya et al., Early Interactions of Rust Pathogen *Puccinia arachidis* (Speg.) with Groundnut Genotypes Varying in Resistance. *International Journal of Bio-resource and Stress Management*, 2022; 13(9), 954-960. HTTPS://DOI.ORG/10.23910/1.2022.3045.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 23rd April 2022 RECEIVED in revised form on 27th August 2022 ACCEPTED in final form on 09th September 2022 PUBLISHED on 21st September 2022

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.), is an important food legume and oilseed crop of the world. The seed is a rich source of oil (35–56%), protein (25–30%), carbohydrates (9.5–19%), minerals (P, Ca, Mg and K) and vitamins (E, K and B, Gulluoglu et al., 2016). Groundnut has many uses in industries of food, feed, paints, lubricants and insecticides (Variath and Janila, 2017). The average productivity in India is low at 1635 kh ha^{-1}compared to that of United States of America (4254 kg ha^{-1}) and China (3906 kg ha^{-1}) (Anonymous, 2020). It is severely constrained by biotic and abiotic factors, including incidence of fungal diseases such as late leaf spot (*Cercosporidium personatum* Berk and Curtis) and rust (*Puccinia arachidis* Speg., Kumari et al., 2014). Together, both the diseases can cause up to 70% yield losses in susceptible cultivars, on which most smallholder farmers often rely in the developing countries (Daudi et al., 2019).

Currently, the disease is managed mainly by growing resistant cultivars and application of fungicides (Daudi et al., 2021). Resistance to rust in groundnut is predominantly governed by homozygous recessive genes even though partially dominant or dominant gene actions have been reported. It is controlled in monogenic, digenic and trigenic manners (Daudi et al., 2021a). Further, resistance to rust has been identified in wild relatives, elite inbred lines, recently developed breeding lines and commercial cultivars (Pande and Rao, 2001, Favero et al., 2015, Sudini et al., 2015, Han et al., 2018, Power et al., 2019).

However, there is limited understanding on the histopathological differences among the genotypes that might have led to the visual differences in signs and symptoms including lesion and sporulation levels. Interactions between diverse species and races of pathogens and plants can elicit compatible or incompatible reactions with complex molecular responses (Andersen et al., 2018). Analyzing the host-pathogen interactions at microscopic level can provide an insight into understanding disease resistance. Histopathological methods have been used to understand the infection processes of plant pathogens on host plants and to investigate the morphological changes during host-pathogen interactions. Among the rust fungi, the early infection processes were well studied in host-pathogen combinations of wheat- *P. striiformis* f.sp. *tritici* (Ma and Shang, 2009, Wang et al., 2010, Zhang et al., 2012, Esmail et al., 2018, Zhao et al., 2019), wheat – *P. triticina* (Wang et al., 2012, Wesp-Guterres et al., 2013), wheat-*P. graminis* (Maree et al., 2020), barley – *P. graminis* (Maree et al., 2020), Coffee –*Hemileia vastatrix* (Ramiro et al., 2009), soybean – *Phakopsora pachyrhizi* (Vittal et al., 2014) and grapevine-*Neophysopella tropicalis* and *N. meliosmae-myrianthae* (Santos et al., 2020). However, such studies characterizing rust resistance in groundnut at cellular and tissue levels are limited. The earliest known histopathological aspects of early infection in groundnut were characterized during the 1980s by Cook (1980), Liang-gao (1987) and Mayee (1987), but since then, little work has been reported. Leal-Bertioli et al. (2010) documented the initial interaction of *P. arachidis* in *A. hypogaea* cv. IAC-Tatu (cultivated, susceptible) and *A. stenosperma* V10309 (wild, resistant) until penetration. Since then, there were no known studies made to understand the histopathological resistance to rust in groundnut. Moreover, the answers to the differences during penetration and post-penetration events in susceptible and resistant groundnut genotypes, if any, are elusive. Understanding such mechanisms responsible for the *P. arachidis* resistance could have implications in groundnut resistance breeding and in disease management.

Keeping these in view, the present study was conducted with the objectives of (i) Observing the early infection strategy of *P. arachidis* in groundnut and (ii) Evaluating the components of resistance against rust for a comprehensive understanding of resistance to rust in groundnut.

2. MATERIALS AND METHODS

The experiment was carried out in the Department of Plant Pathology, College of Agriculture, Rajendranagar, Professor Jayashankar Telangana State Agricultural University, Hyderabad, India (17.3220°N, 78.4023°E) during the June–September 2019. Six groundnut genotypes *viz.,* TMV 2, K-6, ICGV-171015, ICG-11426, ICGV-13229 and GPBD4 representing different levels of resistance were selected based on prior knowledge of their reaction to *P. arachidis* (Janila et al., 2016, Deshmukh et al., 2018, Deshmukh et al., 2020). The seed material was obtained from International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad.

2.1. Inoculation and maintenance of plants

The urediniospores of *P. arachidis* were collected using a cyclone spore collector (Fisher Scientific Co., USA) from infected groundnut plants of susceptible check TMV 2 at ICRISAT. Groundnut plants of each of the six genotypes were grown in plastic pots (7-inch diameter) consisting of sterilized soil mixture of sand and red soil (1:1, w/w) filled up to 3/4th the height and watered until moist. Three seeds per pot were sown equidistantly from one another for each genotype. The pots were maintained in a glasshouse at 26±2°C and watered regularly. Six-week-old groundnut plants were selected for inoculation with *P. arachidis* using a slightly modified detached leaf assay method (Vittal et al., 2014). Fully expanded quadifoliate leaves (third or fourth from the top) were excised through pulvinus. The

terminal part of the petiole was cut using sterile blade. Excised leaves were surface-sterilized by dipping in 1% (w/v) sodium hypochlorite for 1 min, rinsed three times with double distilled water and blotted dry on sterile filter paper. Single quadrifoliate leaf of each groundnut genotype was uniformly inoculated by spraying with freshly collected urediniospore suspension of *P. arachidis* at a concentration of 5×10^4 ml^{-1}. The inoculated leaves were placed in Petri dishes containing 1.5% water agar media. The plates were kept in zip-lock bags and incubated in 12 h of darkness and 12 h of light inside a B.O.D incubator (Vihaan Tech Services, Hyderabad, India). The single detached leaves of each groundnut genotype were considered as the experimental units. The Petri dishes containing detached leaves were arranged inside the chamber in a completely randomized design with three replications.

2.2. Staining and destaining

Five mm diameter leaf discs were randomly excised at 6 h after inoculation (hai), and each day until 5 days after inoculation (dai). Individual discs were stained and fixed by submerging them in a staining solution of 0.05% trypan blue in lactophenol/ethanol (1:2, v/v), boiled for 1 min and incubated at room temperature overnight. For destaining, the discs were immersed in lactophenol/ethanol solution and rinsed in water for many times to remove excess trypan blue (Vittal et al., 2014). Chlorophyll in the leaf discs were cleared by immersing in ethanol/chloroform (3:1, v/v) containing 0.15 % trichloroacetic acid for 15 min at 70°C. The discs were kept immersed in the solution for up to 4 to 5 days until the chlorophyll was completely cleared with periodic changes of clearing solution as and when the solution turned green in color (Vittal et al., 2014).

2.3. Microscopy

The stained leaf discs were examined using an Olympus BX 51 compound microscope (Olympus Corp., USA) at 100x and 400x magnifications for germination, appressoria formation, penetration, hyphal colonization in the intercellular spaces of mesophyll tissue and mesophyll cell death or necrosis at 6 hai and 1, 2, 3, 4 and 5 dai. Images were captured using TCapture software (Fuzhou Tucsen Co., Fujian, China) mounted on the microscope.

3. RESULTS AND DISCUSSION

In this study, an attempt was made to understand the early infection strategy of *P. arachidis* on six groundnut genotypes with varying resistance viz., susceptible (TMV 2 and K 6), moderately resistant (ICGV 13229 and ICGV 171015) and resistant (ICG 11426 and GPBD 4) genotypes.

3.1. Pre-penetration

Adhesion of urediniospores was observed on the surface of groundnut leaves at 6 hai of the suspension. Spore germination was observed at 6 to 12 hai and continued until 24 hai in all the six groundnut genotypes (Figure 1A and 2A). This was similar to the observations made by Leal Bertioli et al. (2010), who studied the ultrastructural differences in the initial interaction of *P arachidis* in *A. hypogaea* (susceptible) and *A. stenosperma* (resistant). They reported that there were no differences in the pre-penetration events of *P. arachidis* between susceptible and resistant genotypes just like the observations made in this study. In the present study, the urediniospores germinated with a single unbranched germ tube measuring 5–6 µm in diameter (Figure 1A and 2A). The lengths of the germ tube varied with most of them ranging between 100–200 µm and some even longer. At or near the stomata, the germ tube swelled to form an appressorium of about similar size to the germinated urediniospore (Figures 1A and 2A). This is in confirmation to the findings of Leal Bertioli et al. (2010) and Cook (1980). Leal Bertioli et al. (2010) reported urediniospore germination, germ tube formation and appressorium formation in both the *A. hypogaea* and *A. stenosperma*. Between 1–2 dai, appressorium facilitated in the attachment of the germinated urediniospore to the stomata. These events constituted the pre-penetration stage (Figures 1A and 2A).

3.2. Penetration

In the penetration stage, a narrow penetration hypha (Figures 1B, C and D) grew from the appressorium traversing the stomata and exited into the mesophyll cell layer. While stomatal penetration was most commonly observed in all the six genotypes, epidermal penetration, though infrequent, was also observed (Figures 2A and 2B). In penetration through epidermis, brown discoloration of the attacked epidermal cell indicating epidermal cell death was observed. A similar observation was made by Vittal et al. (2014) in soybean infected with *P. pacrhyrhizi.* The penetration hypha traversed through the attacked epidermal cell and exited into the intercellular spaces of the meosphyll cells. (Figure 2). The pre- and penetration events summing until now were similar in all the six groundnut genotypes until 2–3 dai irrespective of their varying resistance. This is unlike the observation made by Leal Bertioli et al. (2010) who reported the absence of penetration in the resistant *A. stenosperma* unlike *A. hypogaea* where *P. arachidis* formed penetration hypha and entered inside the host tissue.

3.3. Post-penetration

Histological differences among the genotypes were observed during post-penetration at 3–5 dai. During this, the primary hypha formed from the penetration hypha (Figures 1D-F and 2D-F). In groundnut genotypes TMV 2 (Figure 3A) and K 6 (Figure 3B), the hyphal growth of *P. arachidis*

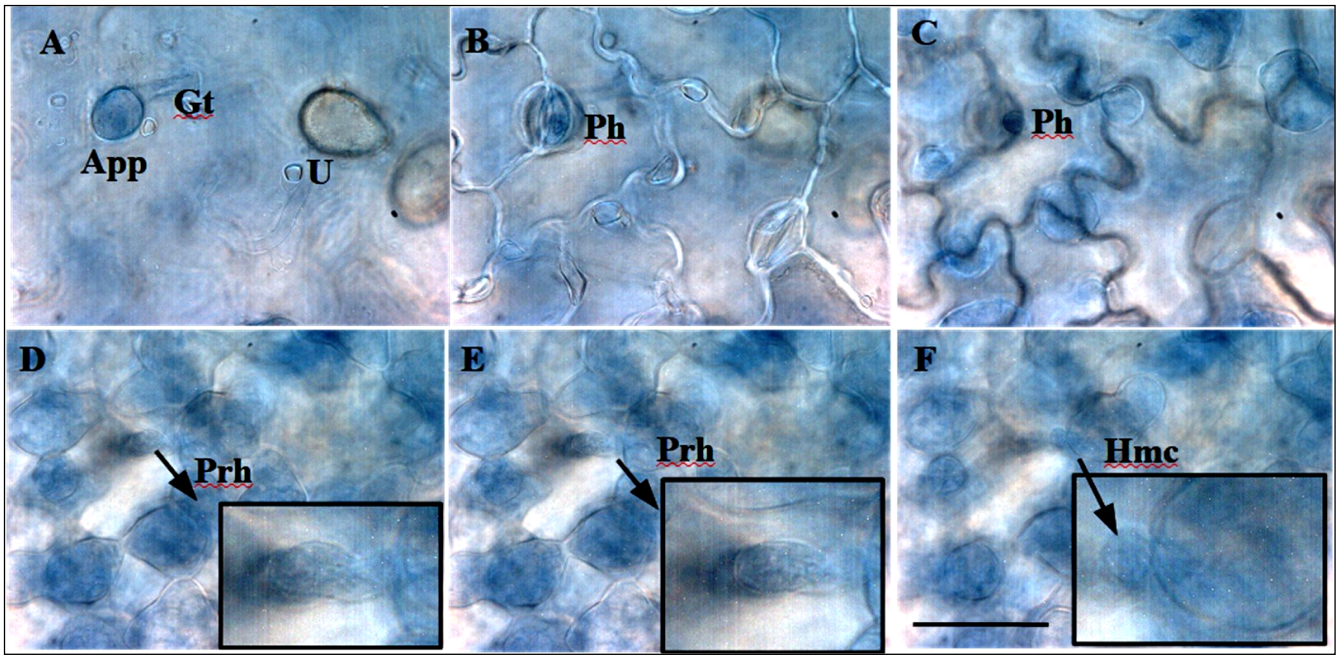

Figure 1: Pre-penetration and penetration of *Puccinia arachidis* through stomata on groundnut variety TMV 2 observed during 1–3 days after inoculation (dai). A. Pre-penetration encompassing germ tube (Gt) and appressorium (App) formation from a single urediniospore (U). B and C. Penetration by formation of penetration hypha (Ph) through the stomata at 1–2 dai. D and E. Primary hypha (Prh, inset) traversing into the intercellular spaces of mesophyll. F. Formation of haustorial mother cell (Hmc, inset) at 3 dai on the mesophyll cells. Leaves were stained with trypan blue and observed with differential interference contrast microscopy using Olympus BX 51 microscope. Scale bar represents 20 µm.

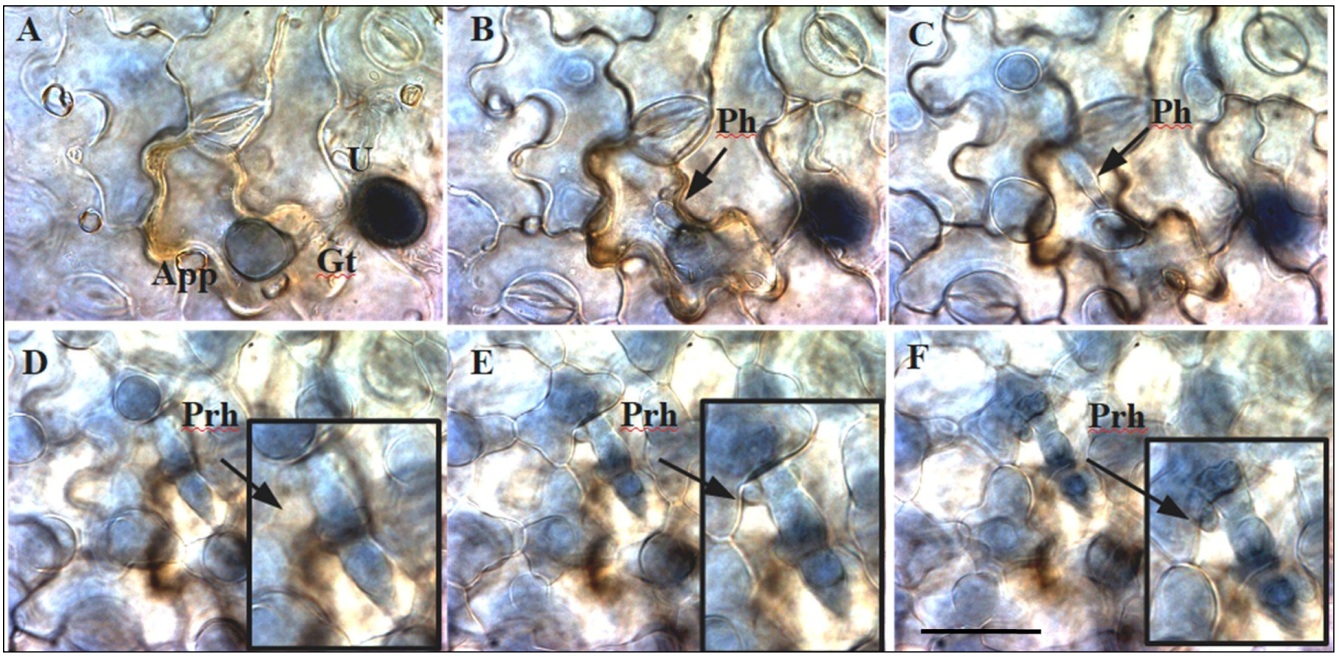

Figure 2: Pre-penetration and penetration of *Puccinia arachidis* through epidermal cell on groundnut variety TMV 2 during 1–3 days after inoculation (dai). A. Pre-penetration encompassing germ tube (Gt) and appressorium (App) formation from a single urediniospore (U). Attachment of the appressorium to the epidermal cell resulting in necrosis (brown discoloration) of the attacked epidermal cell. B. Formation of penetration hypha (Ph, arrow) at the site of appressorial attachment. C. Penetration hypha (Ph, arrow) traversing the epidermal cell. D, E and F. Initiation of Primary hypha (prh, arrow and inset) and traversing across the intercellular spaces of mesophyll at 3 dai. Leaves were stained with trypan blue and observed with differential interference contrast microscopy using Olympus BX 51 microscope. Scale bar represents 20 µm.

expanded through the intercellular spaces of mesophyll cells along with the formation of haustoria in the mesophyll cells. By 4 dai, the colonization of mesophyll tissue involving seven to ten mesophyll cells was observed and by 5dai, extensive colonization expanding 20 to 30 mesophyll cells was observed. Cell death or disintegration was not observed in the mesophyll cells suggesting biotrophic parasitsm of *P. arachidis*, where the pathogen requires the presence of a living host cell for survival. The compatible interaction of *P. arachidis* with the genotypes TMV2 and K 6 indicated their susceptibility to rust disease. It is to note that TMV 2 and K 6 are popular susceptible checks used in rust screening in India (Sudini et al., 2015). In genotypes ICGV 171015 (Figure 3C) and ICGV 13229 (Figure 3D), the fungal growth was sparse and gradual, expanding five to six mesophyll cells at 3-5 dai. Further, disintegration of mesophyll cells corresponding to the intercellular spaces where the hyphae were present suggested the attempts made by these two genotypes to arrest the spread of the hyphae through mesophyll necrosis as visualized by the retention of the trypan blue stain (Vittal et al., 2014). The disintegrated mesophyll cells have deprived *P. arachidis* off the nutrients for survival and growth indicating defense response by these two genotypes. In contrast, the genotypes ICG 11426 (Figure 3E) and GPBD 4 (Figure 3F) showed mesophyll cell death as early as 3 dai and by 4–5 dai, the hyphal growth was ceased due to extensive mesophyll necrosis. Discoloration of mesophyll cells in groups and complete arrest of the hyphal growth indicated incompatible interaction of *P. arachidis* with the genotypes ICG 11426 and GPBD 4 and their resistance to rust disease. The incompatible interaction is in agreement with previously reported resistance nature of ICG 11426 and GPBD 4. Genotype ICG 11426 is a breeding line developed at ICRISAT, India with resistance to rust, early leaf spot and late leaf spot disease (Sudini et al., 2015). GPBD 4 is an improved Spanish bunch groundnut variety with early maturity, high yield, high pod growth rate, desirable pod and kernel features, high oil content and resistance to rust and late leaf spots (Jakkeral et al., 2013).

In view of the frequent breakdown of resistance to foliar diseases as in wheat rust, where the breakdown of the Yr17 resistance gene was reported in cultivars to yellow rust disease caused by *Puccinia striiformis* f.sp. *tritici* (Bayles et al., 2000, El-Jarroudi et al., 2011), the present findings assume significance in identification of sources of resistance to rust disease in groundnut at different stages. For instance, in this study, plant defense response was observed during post-penetration and Leal Bertioli et al. (2010) observed it during penetration where it was reported as an immune reaction. It is possible that there may be more unexplored resistance mechanisms acting at different stages of infection, which when combined can provide plants with

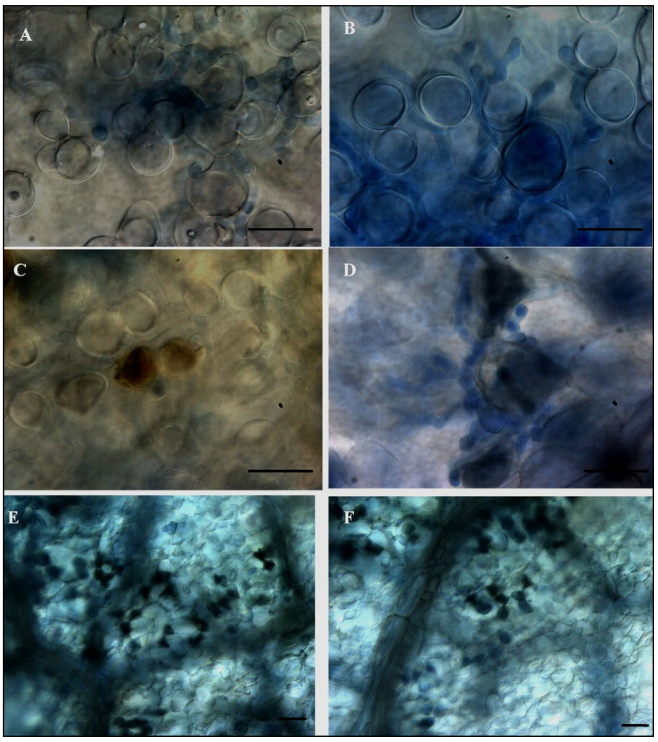

Figure 3: Post-penetration of Puccinia arachidison six groundnut genotypes observed during 3–5 days after inoculation (dai). In the susceptible genotypes TMV 2 (A) and K-6 (B), extensive growth of the fungus in the intercellular spaces of mesophyll and presence of haustoria in the mesophyll cells were clearly seen at 4 and 5 dai. In the moderately resistant genotypes ICGV 17015 (C) and ICGV 13229 (D), two events were observed simultaneously between 3 and 5 dai. While the fungus attempted to spread between the intercellular spaces of the meosphyll cells, the host attempted to arrest the spread of hyphae through initiation of defence as evident by mesophyll cell death (visualized by retention of trypan blue stain). In the resistant genoytpes ICG 11426 (E) and GPBD 4 (F), the growth of P. arachidis inside the mesophyll was arrested by extensive mesophyll cell death visualized by the retention of trypan blue stain at 3 to 4 dai. Leaves were stained with trypan blue and observed with differential interference contrast microscopy using Olympus BX 51 microscope. Scale bar represents 20 µm.

multiple barriers to infection (Rubiales and Niks, 2000). The continuous demand for novel defense mechanisms provides tremendous scope for incorporation of resistance into commercial plant genotypes. More detailed resistance studies on histological mechanisms coupled with molecular, biochemical and physiological investigations can generate valuable information on most promising mechanisms for resistance.

4. CONCLUSION

The study had provided new insight into the early interactions of *P. arachidis* with six groundnut

genotypes at histological level which might explain the differences in the resistance of the genotypes. There were no differences among the genotypes in pre-penetration and penetration events. The study observed (i) compatible interaction with susceptible genotypes TMV2 and K 6; (ii) incompatible interaction with resistant genotypes ICG 11426 and GPBD 4; and (iii) an intermediary interaction with moderately resistant genotypes ICGV 171015 and ICGV 13229.

5. FUTURE RESEARCH

Further studies during these early interaction events at molecular and physiological levels coupled with the histological differences might increase our understanding for different underlying mechanisms of resistance in groundnut to *P. arachidis*.

6. ACKNOWLEDGEMENT

The authors are highly grateful to Professor Jayashankar Telangana State Agricultural Univeristy, Rajendranagar, Hyderabad, India for providing the financial assistance to carry out the research work.

7. REFERENCES

Andersen, E.J., Ali, S., Byamukama, E., Yen, Y., Nepal, M.P., 2018. Disease Resistance Mechanisms in Plants. Genes 9(7), 339–369.

Anonymous. 2020. Statistical data on groundnut area, production and productivity of India, China, United States of America and World [Internet]. Available from: http://faostat.fao.org

Bayles., R, Flath, K., Hovmoller, M., Vallavieille-Pope, C., 2000. Breakdown of the Yr17 resistance to yellow rust of wheat in northern Europe — A case study by the yellow rust sub-group of COST 817. Agronomie 20(7), 805–811.

Cook, M., 1980. Host-parasite relations in uredial infections of peanut by *Puccinia arachidis*. Phytopathology 70(8), 822–826.

Daudi, H., Mathew, I., Rathod, A., Ojiewo, C.O., 2021a. Combining ability and gene action controlling rust ersistance in groundnut (*Arachis hypogaea* L.). Scientific Reports 11(1), 16513–16525.

Daudi, H., Shimelis, H., Mathew, I., Oteng-Frimpong, R., Ojiewo, C., Varshney, R.K., 2021b. Genetic diversity and population structure of groundnut (*Arachis hypogaea* L.) accessions using phenotypic traits and SSR markers: implications for rust resistance breeding. Genetic Resources and Crop Evolution 68, 581–604.

Daudi, H., Shimelis, H., Mwadzingeni, L., Laing, M., Okori, P., 2019. Breeding groundnut for rust resistance: A review. Legume Research 42(3), 291–299.

Deshmukh, D.B., Marathi, B., Sudini, H.K., Variath, M.T., Chaudhari, S., Manohar, S.S., Rani, Ch.V.D., Pandey, M.K, Pasupuleti, J., 2020. Combining high oleic acid trait and resistance to late leaf spot and rust diseases in groundnut (*Arachis hypogaea* L.). Frontiers in Genetics 11(514), 1-15.

Deshmukh, D.B., Chaudhari, S., Marathi, B., Rani, C.V.D., Sudini, H.K., Variath, M.T., Manohar, S.S and Pasupuleti, J., 2018. A non-destructive seed sampling method for high throughput genotyping in groundnut. Journal of Research PJTSAU 46(4), 20-27.

Leal-Bertioli, S.C.M., De Farias, M.P., Silva, P.I.T., Guimaraes, P.M., Brasileiro, A.C.M., Bertioli, D.J., De Araujo, A.C.G., 2010. Ultrastructure of the initial interaction of *Puccinia arachidis* and *Cercosporidium personatum* with leaves of *Arachis hypogaea* and *Arachis stenosperma*. Phytopathology 158(11–12), 792–796.

El Jarroudi, M., Giraud, F., Vrancken, C., Junk, J., Tychon, B., Hoffmann, L., Delfosse, P., 2009. First report of wheat leaf rust in the Grand Duchy of Luxembourg and the progress of its appearance over the 2003–2008 period. Plant Disease 93(9), 971–971.

Esmail, S.M., Omara, R.I., Abdelaal, K.A.A., Hafez, Y.M., 2018. Histological and biochemical aspects of compatible and incompatible wheat- *Puccinia striiformis* interactions. Physiological and Molecular Plant Pathology 106, 120–128.

Favero, A.P., dos Santos, R.F., Simpson, C.E., Valls, J.F.M., Vello, N.A., 2015. Successful crosses between fungal-resistant wild species of *Arachis* (section *Arachis*) and *Arachis hypogaea*. Genetics and Molecular Biology 38, 353-365.

Gulluoglu, L., Basal, H., Onat, B., Kurt, C., Arioglu, H., 2016. The effect of harvesting on some agronomic and quality characteristics of peanut grown in the Mediterranean region of Turkey. Field Crops Research 21, 224–232.

Han, S., Yuan, M., Clevenger, J.P., Li, C., Hagan, A.X.Z., He, G., 2018. A SNP-based linkage map revealed QTLs for resistance to early and late leaf spot diseases in peanut (*Arachis hypogaea* L.). Frontiers in Plant Science 9, 1012-1021.

Jakkeral, S.A., Nadaf, H.L., Gowda, M.V.C., Bhat, R.S., 2013. Inheritance of rust resistance in cultivated groundnut (*Arachis hypogaea* L.) Indian Journal of Genetics and Plant Breeding 73(4), 450-453.

Janila, P., Pandey, M.K., Shasidhar, Y., Variath, M.T., Sriswathi, M., Khera, P., 2016. Molecular breeding for introgression of fatty acid desaturase mutant alleles (ahFAD2A and ahFAD2B) enhances oil quality in

high and low oil containing peanut genotypes. Plant Science 242, 203-213.

Kumari, V., Gowda, M.V.C., Tasiwal, V., Pandey, M.K., Bhat, R.S., Mallikarjuna, N., Upadhyaya, H.D., Rajeev, K, Varshney, R.K., 2014. Diversification of primary gene pool through introgression of resistance to foliar diseases from synthetic amphidiploids to cultivated groundnut (*Arachis hypogaea* L.). Crop Journal 2, 110–119.

Lian-gao, Z., 1987. The groundnut rust situation in the People's Republic of China. Proceedings of a discussion group meeting on groundnut rust disease. 24-28 September 1984; ICRISAT Centre, Patancheru, Hyderabad, Andhra Pradesh, India103–106.

Ma, Q., Shang, H.S., 2009, Ultrastructure of stripe rust (*Puccinia striiformis* f. sp. *tritici*) interacting with slow-rusting, highly resistant, and susceptible wheat cultivars. Plant Pathology 91(3) 597–606.

Maree, G.J., Castelyn, H.D., Bender, C.M., Boshoff, W.H.P., Pretorius, Z.A., 2020. Comparing infection and colonisation of *Puccinia graminis* in barley and wheat. Australasian Plant Pathology 49, 431–445.

Mayee, C.D., 1984. Rust disease of groundnut in Maharashtra state of India. 1987. Proceedings of a discussion group meeting on groundnut rust disease. 24–28 September 1984; ICRISAT Centre, Patancheru, Hyderabad, Andhra Pradesh, India 81–89.

Pande, S., Rao, J.N., 2001. Resistance of wild *Arachis* species to late leaf spot and rust in greenhouse trials. Plant Disease 85, 851-855.

Power, I.L., Tillman, B.L., Brenneman, T.B., Kemerait, R.C., Stevenson, K.L., Culbreath, A.K., 2019. Field evaluation and components of peanut rust resistance of newly developed breeding lines. Peanut Science 46(1), 22–36.

Ramiro, D.A., Escoute, J., Petitot, A.S., Nicole, M., Maluf, M.P., Fernandez, D., 2009. Biphasic haustorial differentiation of coffee rust (*Hemileia vastatrix* race II) associated with defence responses in resistant and susceptible coffee cultivars. Plant Pathology 58(5), 944–955.

Rubiales, D., Niks, R.E., 2000. Combination of mechanisms of resistance to rust fungi as a strategy to increase durability. In: Options méditerranéennes, Serie A: SéminairesMediterranneennes, Numero 40. Durum wheat improvement in the Mediterranean region: New challenges. In: Proceedings of the seminar jointly organized by CIHEAM, Centre Udl-IRTA, CIMMYT and ICARDA. Zaragoza, Spain, 12–14 April.

Santos, R.F., Primiano, I.V., Amorim, L., 2020. Identification and pathogenicity of *Neophysopella* species associated with Asian grapevine leaf rust in Brazil. Plant Pathology 70(1), 74–86.

Sudini, H., Upadhyaya, H., Reddy, S.V., Mangala, U.N., Rathore, A., Kumar, K., 2015. Resistance to late leaf spot and rust diseases in ICRISAT's mini core collection of peanut (*Arachis hypogaea* L.). Australasian Plant Pathology 44(5), 557–566.

Variath, M.T., Janila, P., 2017.Economic and academic importance of peanut. In: Varshney, R., Pandey, M., Puppala, N. (Eds.). The Peanut Genome. Springer, Cham, 7–26.

Vittal, R., Paul, C., Hill, C.B., Hartman, G.L., 2014. Characterization and quantification of fungal colonization of *Phakopsora pachyrhizi* in soybean genotypes. Phytopathology 104(1), 86–94.

Wang, C., Zhang, H., Buchenauer, H., Kang, Z., 2010. Microscopic observation on the development of *Puccinia striiformis* in the spike and stem of wheat. Phytopathology 159(4), 314–316.

Wang, X., McCallum, B.D., Fetch, T., Bakkeren, G., Marais, G.F., Saville, B.J., 2012. Comparative microscopic and molecular analysis of Thatcher near-isogenic lines with wheat leaf rust resistance genes Lr2a, Lr3, LrBor Lr9 upon challenge with different *Puccinia triticina* races. Plant Pathology 62(3), 698–707.

Wesp-Guterres, C., Martinelli, J.A., Graichen, F.A.S., Chaves, M.S., 2013. Histopathology of durable adult plant resistance to leaf rust in the Brazilian wheat variety Toropi. European journal of plant pathology 137(1), 181–196.

Zhang, H., Wang, C., Cheng, Y., Chen, X., Han, Q., Huang, L., Kang, Z., 2012. Histological and cytological characterization of adult plant resistance to wheat stripe rust. Plant Cell Reports 31(12), 2121–2137.

Zhao, Y., Cheng, P., Li, T., Ma, J., Zhang, Y., Wang H., 2019. Investigation of urediospore morphology, histopathology and epidemiological components on wheat plants infected with UV B induced mutant strains of *Puccinia striiformis* f. sp. *tritici*. Microbiology Open, e870.