Int.J.Curr.Microbiol.App.Sci (2020) *9*(5): 370-376

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 5 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.905.042

Pollen-Pistil Interaction among Certain Triploid Derivatives of *Arachis* Species

S. Saravanan* and L. Mahalingam

Tamil Nadu Agricultural University, Vriddhachalam, India

**Corresponding author*

Keywords

Pollen pisltil interaction, Wild groundnut, wide cross, Pollen attrition

Article Info

Accepted:
05 April 2020
Available Online:
10 May 2020

A B S T R A C T

The pollen tube growth in wide crosses involving *Arachis hypogeae* (var VRI 2, TMV 7 and TMV 13) and wild *Arachis sp.* (*Arachis stenosperma*, *Arachis cruziana* and *Arachis kempmercadoi*) have been interpreted among nine crosses. The wide crosses were monitored for pollen attrition and pollen proliferation, being construed that greater levels of genetic distance trigger larger quantum of physiological and physical alterations at various levels of pistil growth. Pollen germination administered greater effect among wide crosses battling poor penetration of pollen tube in the transmitting tissue. The rate of pollen tube proliferation among wide crosses at different levels of pistils was found significant at initial level than at the later stages. The counts of pollen tubes growing down the style were recorded in all the treatment over different level of pistils. The pattern of reduction of tubes at different levels of pistils was very similar for all the crosses. The significantly higher level in proliferation of pollen tubes was noticed in the cross TMV 7 x *Arachis stenosperma*) as 39.50 per cent and is followed by the cross VRI 2 x *Arachis kempmercadoi* (35.25 per cent). The crosses, VRI 2 x *Arachis stenosperma*, TMV 13 x *Arachis stenosperma* and TMV 13 x *Arachis cruziana e*xhibited significantly lower value of pollen tube proliferation at different levels of pistils. The failure of pollen tube to reach and fertilize the ovule is given by pollen attrition. Stylar attrition reported to be maximum in the cross VRI 2 x *Arachis stenosperma* while is low in the crosses, VRI 2 x *Arachis kempfmercadoi*, TMV 13 x *Arachis kempfmercadoi* and TMV 7 x *Arachis stenosperma.* It was evidenced that significant reduction in density of pollen tubes observed as they grow along the style. Also, the studies on genetic system of pollen pistil interaction construe more obvious in the upper part of the style than in the lower half delivering unique physical or physiological confronts impacting significant negative interaction over genetic systems of the gametophytes.

Introduction

Post pollination processes in plants always triggered by the attitude of inter and intra sexual mechanism adopted. In plants, judicious counts of sex gametophytes generated during the gametophytic phase. Nevertheless, part of lesser numbers of female gametophytes attains successful fertilization. Pollen grains on compatible stigma germinate and produce a pollen tube from which the sperm cells are migrated to the female gametophyte through the pollen tube transmitting tissue holding indiscriminate territory between stigma and ovary.

Pollen attrition prelude compatible action thereby targets productive number of ovules than that of seeds (Saravanan, 2017). The greater the pollen source, the higher the seed set, seed germination and seedling vigor while also the pollen thickness increased upon the length of exposure to pollinators varying with time to time and from population to population (Lankinen *et al.,* 2018). Pollen density along with thickness always adjudged to stimulate positive interaction between pollen and stigma. The pollen competition is eventually a result of pollen loads on the stigma. (Zheng *et al.*, 2018). Erstwhile, rate of deposition of pollen grains on stigma exceeds the ovule production for fertilization (Bedinger *et al.,* 2017).

Anyhow, the candidate genes ultimately resolve the unifying or integrating several steps constituting successful fertilization of embryo. Further, elusive molecular characterization after triggering genetic diversity could aid fruitful for proven and desirable crop improvement (Rajendra Kumar *et al.,* 2017). In *Arachis*, seeds per pod are highly interfered by the pollen competition along with the vigour of the resultant sporophyte. The pollen tubes have been appraised to follow a fixed pattern of reduction since the initial number of pollen tubes is progressively reduced along the style. Reduced growth of pollen tube might have resulted through an interaction effect of pollen and style interaction. Therefore, integrative steps involved in the interaction of pollen and pistil plays an effusive role during fusion of the sex gametes.

Materials and Methods

The study was made using multiple sets of wide crosses at Regional Research Station, Vriddhachalam. The treatment involved in the dusting of different loads of pollen from different sets of diploid *Arachis species* (*Arachis stenosperma* and *Arachis kempmercadoi*) to stigma of tetraploid *Arachis hypogaea* (var VRI 2, TMV 7 and TMV 13) parent. Flowers were collected at time of anthesis and rubbed over the stigma of female parent that had been emasculated at previous day by removing the anthers and petals to make them unattractive to insects (Veerappan *et al.*, 2014).

Among the crosses, five pistils were collected and fixed in formalin: acetic acid: ethanol in the ratio of 1:1:18. After 3 days after pollination, when the pollen tube growth might have reached the base of the style, the pistils were fixed in the fixative. From squash preparation of pistils washed in distilled water, the pollen tube growth along the style was monitored after the pistils have boiled in 5 per cent sodium sulphate to soften tissues and stained with 0.1 per cent aniline blue in 0.1N K_3PO_4 (Geetha and Jayaraman, 2000 and Geetha *et al.*, 2004).

The pre-treated pistils were kept under Nikon microphot-Fx microscope with fluorescence attachment, illuminated with 200w high pressure UV lamp for examination. The observations were taken with B (380-490nm) and or BG (650nm) excitation filters in combination with BA 520 or BA 530 barrier filter. Colour photographs were taken either with barrier filter (greenish yellow) or without barrier filter (blue background with bright white fluorescing pollen tube). Data were analyzed in completely randomized design and treatment means were compared with Duncans Multiple Range Test.

Results and Discussion

The proportionate interpretation on key phenological traits of parents and triploid derivatives of *Arachis* was given in Table 1. Progenies of different genetic nature construed significant variations in habit, leaf texture, leaf shape, bract shape, bract size, length of staminal column and pollen fertility. The compatibility mechanism among the wide crosses is adjudged through perturbation of pollen germination, pollen tube growth and fertilization which are determined by fluorescence microscopy. The fluorescence microscopic technique involve pistils getting stained with aniline blue and better viewed under ultra violet illumination. The callose plugs present in the pollen tube fluoresce brightly thereby the pollen tube can be clearly interpreted in the stylar tissue and documented. Further insufficient softening of pistils leaves the specimen hardy and difficult to observe the pollen tube growth due to differential refraction. Moreover, also the over softening may result in breakage of pistils during gentle press and render the observation of pollen tube faulty. The softening of the pistils of diploid parents was optimum at 12N and 10N NaOH for 6 and 8 hours duration respectively, besides the tetraploids had 10N NaOH for 6 hours duration as optimum. The optimum staining and better fluorescence was obtained when 0.25 per cent aniline blue was dissolved in 0.1N K_3PO_4 and kept for 6 hours.

The studies were made on the few wide crosses of *Arachis* to interrogate the knock of alien pollen on stigma for reviving the fertilization barriers attributed in coordination with pollen germination and its walk on stigma of alien species. Congregate of alien pollen from wild *Arachis species* upon *Arachis hypogeae* recorded poor pollen fertility and germination values compared to parental genotypes (Table 1).

Different forms of distant crosses offered varied proportions of pollen deposition. The wide crosses, VRI 2 x *Arachis kempfmercadoi* and TMV 7 x *kempfmercadoi* recorded higher pollen germination of 38 and 34 per cent respectively. The activity of pollen over the stigma and style of female plant offered scope for interpretation of pollen attrition. Significant variation on growth of pollen grains over the stigma pertaining to different crosses deliberated uneven penetration of pollen tubes over the transmitting tissue along the first half of the stylar canal. This observable variation of pollen tube spread on transmitting tissue of stylar canal registered significantly higher at initial level compared to the later stage of stylar penetration. The gradual reduction in density of pollen tube growing through the stylar canal was observed among all crosses spreading over different levels of pistils.

The significantly higher level in proliferation of pollen tubes was noticed in the cross VRI 2 x *Arachis kempfmercadoi* as 41.31 per cent and is followed by the cross TMV 7 x *kempfmercadoi* (37.65 per cent). VRI 2 x *Arachis stenosperma* (23.69 per cent), TMV 13 x *Arachis stenosperma* (26.25 per cent) and TMV 13 x *Arachis cruziana* (29.48 per cent) *e*xhibited significantly lower value of pollen tube proliferation at different levels of pistils (Table 3). Stylar attrition was determined from the pollen tube not reaching the ovary. Stylar attrition was minimum in the crosses VRI 2 x *Arachis kempfmercadoi* (58.69 per cent) and TMV 7 x *Arachis kempfmercadoi* (62.35 per cent) while VRI 2 x *Arachis cruziana* (76.20 per cent) and TMV 7 x *Arachis cruziana* (73.84 per cent) registered the maximum (Table 4). A dramatic diminution in the pollen tube density along the stylar canal was adjudged.

The data signified the proportionate decrease in pollen tube in the stylar region as compared to opening number of pollen tube at the mouth of stylar canal. This further evidenced the impartial decrease in density of pollen tube all along the stylar region suggesting the possibility of genetic interactions on regulation of pollen tube attrition (Regel, 2019). The greater decrease in pollen tube number at mid stylar region could enhance alarming probability of pollen attrition (Table 3).

Table.1 Morphological dissection of triploids

Pedigree	Length of Primary branch (cm)	Length of Secondary Branch (cm)	Leaf Area (cm^2)	Length of calyx tube (cm)	Length of Standard petal (cm)	Width of Standard petal (cm)	Leaflet colour
VRI 2 x *Arachis kempffmercadoi*	41	60	9.03	5.20	1.32	1.67	Light Green
VRI 2 x *Arachis cruziana*	40	61	8.40	5.30	1.31	1.64	Light Green
VRI 2 x *Arachis stenosperma*	40	58	7.79	5.00	1.29	1.69	Light Green
TMV 7 x *Arachis stenosperma*	39	62	10.08	5.20	1.28	1.58	Light Green
TMV 7 x *Arachis kempffmercadoi*	40	61	8.20	5.00	1.32	1.60	Light Green
TMV 7 x *Arachis cruziana*	40	60	9.89	5.20	1.31	1.55	Light Green
TMV 13 x *Arachis stenosperma*	37	59	8.40	5.40	1.27	1.60	Dark green
TMV 13 x *Arachis kempffmercadoi*	39	58	8.17	5.30	1.29	1.57	Dark green
TMV 13 x *Arachis cruziana*	42	60	9.66	5.10	1.30	1.56	Dark green
VRI 2	45	58	8.30	5.64	1.40	1.77	Light green
TMV 7	42	59	7.82	5.42	1.39	1.69	Light Green
TMV 13	44	57	8.30	5.70	1.35	1.60	Dark Green
Arachis cruziana	38	60	8.52	5.30	1.20	1.30	Light Green
Arachis stenosperma	36	61	7.52	5.10	1.22	1.32	Light Green
Arachis kempffmercadoi	37	62	7.88	5.25	1.18	1.28	Light Green

Table.2 Proportion mean ± SE of pollen grain deposition over stigma

Treatment	Pollen grains in stigma	Germinated pollen grain	Per cent of pollen germination
VRI 2 x *Arachis stenosperma*	1354^{cd}	325^{d}	**24^{d}**
TMV 7 x *Arachis stenosperma*	865^{ef}	225^{e}	**26^{d}**
TMV 13 x *Arachis stenosperma*	1481^{c}	400^{cd}	**27^{cd}**
VRI 2 x *Arachis cruziana*	740^{g}	288^{de}	**20^{bc}**
TMV 7 x *Arachis cruziana*	820^{f}	250^{de}	**25^{d}**
TMV 13 x *Arachis cruziana*	795^{g}	220^{e}	**22^{c}**
VRI 2 x *Arachis kempffmercadoi*	802^{f}	305^{de}	**38^{c}**
TMV 7 x *Arachis kempffmercadoi*	1250^{d}	425^{c}	**34^{c}**
TMV 13 x *Arachis kempffmercadoi*	990^{e}	325^{d}	**24^{d}**
VRI 2	1680^{b}	1124^{ab}	**67^{ab}**
TMV 7	1845^{a}	1352^{a}	**73^{a}**
TMV 13	1695^{b}	980^{b}	**58^{b}**
Arachis stenosperma	954^{e}	440^{c}	**46^{bc}**
Arachis cruziana	658^{gh}	215^{e}	**33^{c}**
Arachis kempffmercadoi	**821^{ef}**	**324^{d}**	**39^{c}**

Means followed by a common letter are not significantly different at 5 per cent by DMRT

Table.3 Mean ± SE of pollen tubes at different levels of pistil

Treatment	Pistil level						Per cent Pollen tube survival
	Stigma	Transmitting tissue	¼ style	½ style	¾ style	Ovary	
VRI 2 x *Arachis stenosperma*	325±5.70	121±2.12	111±1.94	100±1.94	87±1.52	77±1.36	**23.69**
TMV 7 x *Arachis stenosperma*	225±3.95	142±2.49	130±2.28	118±2.28	102±1.79	82±1.44	**36.44**
TMV 13 x *Arachis stenosperma*	400±7.02	163±2.87	150±2.62	136±2.62	117±2.06	105±1.84	**26.25**
VRI 2 x *Arachis cruziana*	295±5.25	135±2.37	120±2.33	108±1.89	102±1.78	99±1.60	**33.56**
TMV 7 x *Arachis cruziana*	288±4.35	115±2.20	98±1.70	91±1.56	88±1.54	80±1.40	**33.33**
TMV 13 x *Arachis cruziana*	250±4.52	132±2.30	95±1.65	87±1.51	82±1.43	79±1.33	**29.48**
VRI 2 x *Arachis kempfmercadoi*	220±5.35	160±2.81	152±2.67	138±2.42	131±2.30	126±2.21	**41.31**
TMV 7 x *Arachis kempfmercadoi*	425±7.46	192±3.37	184±3.23	170±2.98	164±2.88	160±2.81	**37.65**
TMV 13 x *Arachis kempfmercadoi*	325±5.69	137±2.40	130±2.40	118±2.06	103±1.81	84±1.47	**32.70**
VRI 2	1124±19.72	1085±19.04	1002±17.58	992±17.41	968±16.99	952±16.70	**84.70**
TMV 7	1352±23.72	1286±22.57	1229±21.57	1209±21.21	1189±20.86	1156±20.28	**85.50**
TMV 13	980±17.20	895±15.70	821±14.41	796±13.97	728±12.77	702±12.32	**71.63**
Arachis stenosperma	440±7.72	385±6.76	317±5.56	296±5.19	256±4.49	240±4.21	**54.55**
Arachis cruziana	324±5.69	250±4.39	194±3.40	159±2.79	144±2.53	132±2.32	**40.74**
Arachis kempfmercadoi	**405±7.12**	**365±6.40**	**320±5.62**	**260±4.85**	**235±4.40**	**205±3.85**	**50.62**

Means followed by a common letter are not significantly different at 5 per cent by DMRT

Table.4 Pollen attrition per cent for different wide crosses

Treatment	Pistil distribution over stigmatic surface	Pistil distribution inside ovary	Pollen attrition
VRI 2 x *Arachis stenosperma*	325^{d}	77^{gh}	**76.20**
TMV 7 x *Arachis stenosperma*	225^{e}	91^{f}	**63.56**
TMV 13 x *Arachis stenosperma*	400^{cd}	105de	**73.84**
VRI 2 x *Arachis cruziana*	295de	99^{f}	**66.44**
TMV 7 x *Arachis cruziana*	240^{ef}	80^{fg}	**66.67**
TMV 13 x *Arachis cruziana*	268^{e}	79^{fg}	**70.52**
VRI 2 x *Arachis kempfmercadoi*	305de	126^{d}	**58.69**
TMV 7 x *Arachis kempfmercadoi*	425^{c}	160^{d}	**62.35**
TMV 13 x *Arachis kempfmercadoi*	315^{d}	84^{f}	**73.33**
VRI 2	1124 ^{ab}	952^{ab}	**15.30**
TMV 7	1352^{a}	1156^{a}	**14.50**
TMV 13	980^{b}	702^{b}	**28.37**
Arachis stenosperma	440^{c}	240^{c}	**45.45**
Arachis cruziana	324^{d}	132^{d}	**59.26**
Arachis kempfmercadoi	**405^{cd}**	**205^{c}**	**49.38**

Means followed by a common letter are not significantly different at 5 per cent by DMRT

The same results were also reported by Subramanian and Muthiah (2000), Abdelgadir *et al.*, (2012), Pandey *et al.*, (2009) in redgram and Saravanan (2017) in Groundnut. Mazer *et al.*, (2016) concluded pollen attrition was dictated by style length that influences the rate of pollen grain germination *via* stigma clogging or pollen-pollen interactions.

Reports on higher rate of pollen tube attrition in the terminal part of the pistil as pronounced by Cruzan (1989) besides the variation in pollen tube density in the stylar canal pointed by Hormaza and Herrero (1996) and Choudary *et al.*, (2015).

Also it was adjudged the independency of germinating pollen grains on the stigma in the lower part of the pistil. In the present study also, the superimposition of pollen pistil interaction on genetic nature could attribute stylar inhibition of pollen attrition on the stylar region as substantiated by Bedingar *et al.*, (2017). However, the parental cultures exhibited admissible pollen germination and pollen tube growth attributing better seed set besides the tetraploid groundnut shown highest pollen tube proliferations. To conclude, among wide crosses, poor pollen germination lead unprivileged pollen tube proliferations at varied regions of pistils eventually lead to reduced seed set.

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How to cite this article:

Saravanan. S. and Mahalingam. L. 2020. Pollen-Pistil Interaction among Certain Triploid Derivatives of *Arachis* Species. *Int.J.Curr.Microbiol.App.Sci.* 9(05): 370-376.
doi: https://doi.org/10.20546/ijcmas.2020.905.042