Predominace of F1s Sterility Occur in Many Crosses of Vigna Species

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Abstract-In this study investigating the possibility of developing interspecific fertile hybrids between *Vigna radiata* with other wild *Vigna* species to produce durable resistance against Mungbean Yellow Mosaic Virus and bruchids resistant genotypes in the greengram. Among the wild *Vigna* species, *Vigna. umbellata* having durable MYMV resistance and bruchids resistance in *Vigna vexillata* To eradicate MYMV problem in *Vigna radiata*, the *Vigna umbellata* involved to make a more number of crosses with greengram and *Vigna vexillata.* Recovery of fertile F1s is highly challengeable in *Vigna radiata* with other wild Vigna species crosses. Crosses among wild species with cultivated are unable to easily generate fertile hybrids. Even though no crossability barriers problem occur in a matting time. But both level of F1s of fertile and sterile hybrids were produced in *Vigna radiata x Vigna umbellata* and *Vigna radiata x Vigna vexillata* crosses with more number of accession used. The crossability percentage may varied depends on close relation of wild species with greengram. To identify the reproductive barriers blocking the pod development and seed set in in F1s, pollen viability and pistil acceptability and receptivity of same flower was studied in F1s. Pollen-pistil interactions in direct and reciprocal crosses of *Vigna radiate, Vigna umbellata and Vigna vexillata* were investigated to characterize pollen-stigma compatibility. Premature surface portions of stigma cells and pollen were observed to reveal cytological mechanism regulating pistil receptivity and the pistil and pollen interactions. We observed that stigma receptivity, pollen viability and varied depending on the species.

Various reciprocal crosses showed different pollen-stigma in compatibilities expressed their pollen germination rates compared to direct crosses. Some of the cross pollen grains germinated on female stigmas, the pollen tubes were arrested in the pistils and pollen tubes not able to reach the ovaries which could not carried out backcross further. So the embryo sacs remained unfertilized until degenerating. The F1s were affected by pre-fertilization barriers. Pollen pistil incompatibility always towards low pollen fertility and poor stigma receptivity and acceptability are indicating the two factors affecting hybrid set.

Stigma fluids are not favorably help to pollen germination of the same flower, stigma acceptability and receptivity were inhibited the pollen tube growth. Searching actual reason for all the crosses of F1s sterile the detailed pre fertilization barrier and meiotic studies carried out both male and female sterility. Pre fertilization barriers due to Male and female sterilities. Male and female sterilities due to meiotic irregularities happened in F1s. Male sterility due to non separation of tetrad and formation many kind of valents like mono valents, trivalents, tetravalent and multivalent etc., and female sterility due to degeneration occur during megaspore development.

These findings may be helped for wild of *Vigna* species hybridizations beginners. This problem easily overcome the by the more number of accessions are involving in the crossing programme. This is one of the methods to overcome reproductive barriers and may also be useful for clarifying the phylogenetic relationships among Vigna species cultivars with differing phenotypes. Identify the closely wild related proginator of particular species.

Key words: Pre fertilization barrier –*Vigna radiata* – *Vigna umbellata*- *Vigna vexillata*- Pollen failed on stigma – Poor stigma receptivity

INTRODUCTION

New species or genotypes are being evolved through natural crosses which took several years. Many species evolutes naturally by the help of wind and honey bees pollination. Some of the known valuable characters which must to be transferred to one species to another are highly difficulties. While crossing breeder can always met difficulties to achieve the goal**.** Desirable traits have been improved in the existing genotypes by incorporation of desirable genes from genetic resources. "Hybridization breeding" is the best way to introduces valuable traits into existing cultivars for developing new varieties. The biotic or abiotic stress tolerance traits maximum available in breeding stocks or wild progenitors which will be transferred by intra-specific or inter-specific crossing. The variability is very limited among the varieties, so breeders can get the voluble wide genetic resources in other species; breeder can go for inter-specific crosses is expected to refined to further development of crop improvement programs but they are getting barrier problems like pre and post fertilization.

Majorly two types of reproductive barriers like pre- (before fertilization) and post (after fertilization) barriers. Pollination to the other species is completely restricted due to their geographic habitat, structure of the flower like shape and colour , duration of flowering time or pollen carriers or mediator, which is concern with pre fertilization barriers (Lowry *et al*. 2008).

In the inter-specific crosses, some time incompatibility is system also play a major role to inhibit the pollen tube germination or growth in the stylar portion of the flower. Incompatibility in interspecific crosses created post-zygotic barriers between pollen and stigma interaction (Dresselhaus *et al*. 2011).

In interspecific crosses pollen germination in stigma and fertilization with eggs are successful, embryo abortion may happed or un expected abnormal growth of endosperm or over growth of endosperm ie fast proliferation of endosperm which interfere the normal seed development are recorded as post zygotic barriers. The abortion of embryo is leads to abnormal endosperm. Most of the interspecific cross combination failed to develop zygote followed by endosperm development depends on the parental species of plody level (Kinoshita 2007).

Hybrid sterility formed by epistatic interactions between nuclear genes and mitochondrial genome in maternal parent, termed as cytoplasmic male sterility (CMS), which leads to post-zygotic barrier. CMS Male sterility is useful for F1 hybrid seeds production (Yamagishi and Bhat 2014). hybrid sterility or CMS in rice responsible for several genes (Fujii and Toriyama 2008).

Polyploidy levels are available in wild and cultivated crops which is help to increase of genetic diversity (Innan and Kondrashov 2010).

Auto polyploidy developed by multiplication of genome of single species which leads to increasing of genome size and multiplied genes can give useful traits, like big growth of fruit size by increase of cell size (Lavania *et al*. 2012).

Allopolyploid species are formed by merging genomes from

Two or more distantly related species joined to gather to form allopolyploid with divergent species to develop heterozygosity (Chen 2010).

Heterozygosity always increase genetic diversity and phenotypic variability. These two aspect changes rearrangements of genome through multivalent, homoeologous chromosome recombination, elimination of chromosome, and alteration of transcriptome. This study is discussing about the overcome the reproductive barriers

MATERIALS AND METHODS

The genotypes of mungbean and other *Wild Vigna species* used as male and female parents for synthesis of interspecific hybrids are furnished in Table. 1.

Table 1. Name of the *species* selected for hybridization and their special features

Sl.No	Species	Special features	Origin
1.	*Vigna radiata*	Short duration cultivated species	Tamil Nadu
2	*Vigna umbellata*	Immune to MYMV and resistance to bruchids	Himalayan region
3	*Vigna vexillata*	Lengthy pod, high number of seeds per pod & bruchids resistance	Kodaikanal hills

Vigna radiata and six wild *Vigna species* were raised during Rabi 2015-2016 in a crossing block at Agricultural Research Station, Virinjipuram, Vellore. The direct crosses were effected following the method suggested by Boling *et al. (*1961) for hybridization.

The media standardized by in order to investigate the effect of stigmatic fluids/ exudates on pollen germination in the interspecific crosses, experiments were carried out by supplementing the standardized pollen germination media with the extracts of stigma from the corresponding female parent representing each cross combination.

The extract was prepared by grinding 50 mg of stigma in one ml of distilled water and added to 25 ml of the media as suggested by Dundas 1990.The slides were photographed using built in camera of the OLYMPUS microscope.

ARTIFICIAL CROSSES

Direct and reciprocal crosses were made for six combinations *Vigna radiata x Vigna umbellata, Vigna radiata x Vigna vexillata* , and vice versa. A minimum of 80 to 150 flowers from each maternal parent were emasculated and covered with butter paper cover. Next day morning pistils were pollinated using fresh pollen grains collected from paternal parent flowers of respective crosses to be done, male parents also covered with butter paper bags before anthesis. All the pollinated flowers were subsequently bagged with butter paper cover.

We have used very successful method that is BK medium for pollen germination (containing 100 mg $^{-1}$ KNO_3, 200 mg $^{-1}$ $MgSO_4$ $7H_2O$, 100 mg $^{-1}$ H_3BO_3 and 300 mg $^{-1}$ $Ca(NO_3)_2$ $4H_2O$) at Type 1 method the pollen is collected from the flower (one day before blooming) with soft brush. The pollen grains were put into glass slides for germination and subsequently incubated at 30°C for 4 hrs. Type 2 pollen collected from fully opened flowers at morning 8 to 10 AM before anthesis in F1s and parental flowers.

Parents pollen grains that produced a pollen tube. In the F1 hybrids not produced any pollen tubes. The fertile pollen grains formed pollen tubes in stylar portion by taking picture through microscope (Olympus, Tokyo, Japan). The entire optical field consisted of at least 25 pollen grains. The rate of germination was also calculated as the number of germinated pollen grains divided by the total number of pollen grains. In the F1 plants zero pollen germination counted.

Both direct and reciprocal cross combination, some of the cross has not produced any pods in F1s. To know the pollen-pistil interactions in the various cross combination of Vigna species. Samples were collected from two crosses hybrids namely *Vigna radiata x Vigna umbellata* and *Vigna radiata and Vigna vexillata* this observation was insisted to study the pollen-pistil interaction. Pistil receptivity was tested by manual pollinations with fresh fertile pollen grains of female parent. The pollen dusting was done at 08:00–10:00 AM in both F1s and Parents flowers. Out of 10 pistils, five pistils were taken for analysis. The pollinated pistils was allowed for 8 h and then immediately pistil was fixed in FAA solution (90 formalin/5 alcohol/5 glacial acetic acid as volume) and observation was taken by using an fluorescence microscope (OLYMPUS microscope.). For counting the germinated pollen grains used by aniline blue staining method. Pistil receptivity was calculated based on the ratio of the number of pollen grains germinated in the stigma divided by the total number of pollinated pollen grains. Ten pistils were tested at each type and the experiment was repeated three times.

DATA ANALYSIS

Data were analyzed by a two-way analysis of variance (ANOVA) using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) for Windows. Duncan's multiple range test was adopted to determine significant differences between means ($P < 0.05$ or 0.01). The data are presented as the mean value ± standard deviation.

RESULTS

Vigna radiata and three wild *Vigna species* were raised during Rabi 2016-2017 in a crossing block at Agricultural Research Station, Virinjipuram, Vellore. The direct crosses were effected following the method suggested by Boling *et al. (*1961) for hybridization. The media standardized by in order to investigate the effect of stigmatic fluids/ exudates on pollen germination in the interspecific crosses, experiments were carried out by supplementing the standardized pollen germination media with the extracts of stigma from the corresponding female parent representing each cross combination. The extract was prepared by grinding 50 mg of stigma in one ml of distilled water and added to 25 ml of the media. The slides were photographed using built in camera of the OLYMPUS microscope.

ARTIFICIAL CROSSES

Direct and reciprocal crosses were made for four combinations: , *Vigna radiata x Vigna umbellata, Vigna radiata x Vigna vexillata* , and vice versa. A minimum of 80 to 150 flowers from each maternal parent were emasculated and covered with butter paper cover. Next day morning pistils were pollinated using fresh pollen grains collected from paternal parent flowers of respective crosses to be done, male parents also covered with butter paper bags before anthesis. All the pollinated flowers were subsequently bagged with butter paper cover (Pandiyan *et al*.2010 and 2012).

The pollen grains were collected from the flowers previous days evening with a soft brush. The pollen grains were put into glass slides for germination and subsequently incubated at 30°C for 4 h. Parents pollen grains that produced a pollen tube. In the F1 hybrids not produced any pollen tubes. The fertile pollen grains formed pollen tubes in stylar portion by taking picture through microscope (Olympus, Tokyo, Japan). The entire optical field consisted of at least 25 pollen grains. The rate of germination was also calculated as the number of germinated pollen grains divided by the total number of pollen grains. In the F1 plants zero pollen germination counted.

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DISCUSSION

The present investigation was indicating focussed on the rate of pollen tube growth and abnormalities of foreign pollen effect on the cultivated pistils to understand how the reproductive isolation barriers operate on pollen tube penetration in different stages of pistils. The divergent relative species namely *V. umbellata* confers more durable resistance to insect pests and diseases (Watanasit and Pichitporn 1996, Pandiyan et al 2010, 2012) and premkumar et al 2007.. In the present study, low pod set was observed in *V. radiate* ×*V. umbellata* and *V. radiata* × *V. vexillata* crosses. However the pollen fertility was found to be normal. Low pod set observed in the above crosses could be due to the operation of pre-fertilization barriers. Similar results were noticed in the inter-specific crosses of *Vigna* species (Umamaheshwari 2002 and Pandiyan *et al* 2010, 2012).

Basavaraj et al. 2018 results revealed that highest crossability per cent was recorded in the crosses Yellowmung × KBR-1 (17.30 %), DGGV-2× RBL-35 (16.0%), Selection-4×KBR-1 (11.80%), Chinamung × KBR-1 (11.0%) and BGS-9× RBL-35 (10.20%) which were considered as successful crosses. This suggests that the parents of these four interspecific cross combinations may be ideal for transfer of useful genes across the two divergent species and to broaden the genetic base of interspecific hybrid and further, the existence of moderate hybrid pollen fertility (78.24%) coupled with moderate seed germination percentage (36.84%),

Basavaraj et al 2018.The strong pre-fertilization barriers were present in the cross between V. radiata x V. umbellata. Growth and lethality of the interspecific hybrid seedlings were influenced by the genotypes of both parental species.

Prithviraj and Niranjana Murthy 2018. Twenty eight interspecific hybridization involving four mungbean (Vigna radiata) and seven rice bean (Vigna umbellata) varieties were attempted to study crossability and other related parameters. Among twenty eight interspecific crosses attempted sixteen crosses were successful in setting pods. Of these sixteen interspecific crosses, KKM-3 × KBR-1 was found better with high crossability per cent, high hybrid pollen fertility, high seed germination percentage, lower hybrid lethality and lower hybrid breakdown was observed, suggesting that among all the cross combinations attempted this cross combination is a potential source for obtaining new gene combinations. The other two crosses which were next better combinations were KKM-3 × RBL-6 and KKM-3 × BRBM 127

Gopinathan et al. 1986 revealed delay/absence of divisions in the endosperm and the failure of embryo to divide were the post-fertilization barriers responsible for somatoplastic sterility in normal crosses which yielded a few hybrid seeds.

Bassiri *et al* 1986 germination of pollen grains and growth of pollen tubes were studied to determine the cause of barreness in crosses among annual *Cicer species*. In vigna species Pandiyan *et al*. 2010 and 2012. Pollen tube growth was characterized by irregularly spaced and intermittent callose deposits. Failure of seed formation in interspecific pollinations may be attributed to the slowness of pollen tube growthHybrid sterility and Hybrid breakdown observed in vigna species Pandiyan et al. 2010 and 2012 In *V. radiata* × *V. umbellata* cross, the pre-fertilization barriers at all levels of tissue were significant, as indicated by moderate pollen tube penetration and growth

The slow rate of pollen tube growth was noticed in inter-specific crosses of rice (Sarker *et al.* 1983) and maize (Manickam 1996). Although the pollen tube growth was slowed down by the structural aberrations like stigmatic exudation and degeneration of pollen tubes eventually pollen tube penetrated into the bottom stylar tissues and reached the ovary. Similar types of structural malformations were reported in Cotton (Gunasekaran 1997). Umamaheshwari (2002) reported low percentage of pod setin the same cross using different accessions of parents. The techniques such as pollen mixture, application of growth hormones and protoplast fusion may rectify the defects before fertilization and lead to development of new inter-specific hybrids from related species.

The pre-fertilization barriers were predominant in *V. mungo* × *V. umbellata* cross and operated.

Bharathi et al 2006.Preferilization Barrier In Vigna Radiata X Vigna wild species . Chaisan et al. 2013.

In all the stages as delayed or inhibited penetration of pollen tube growth and disorientation of pollen tubes in stylar tissues. The pre-fertilization barriers were not restricted to any one particular stage of development, but had operated very gradually and mildly in varying degree. The common pre-fertilization barriers found to occur in many wide crosses are pollen-pistil incompatibility. These barriers are influenced by delayed pollen grain germination and pollen tube growth of one species on the stigmas of another species (Monika *et al.* 2005, Hodnett *et al.* 2005).

Ganesh *et al*., 2008 reported in cotton pollen germination to an extent of 54% was observed within 2 HAP. Juanzi et al., 2008 reported using immune enzyme and immuno gold labeling techniques and an anti-IAA monoclonal antibody, Li-Jia Qu *et al* 2015 recorded these pre-zygotic hybridization barriers require intensive communication between the male and female reproductive cells and the necessity to distinguish self from non-self interaction partners. It is understood that proper recognition of protein substances, pollen tube penetration on the stigma and co-ordination between the pollen and pistil proteins are necessary to effect normal pollen tube growth. When pollen grains succeed in crossing the stigmatic surface, growing pollen tubes are guided to the micropyle by signals originating in the style and embryo sac (Lord and Russell 2002). During discordant situations, normal metabolism of the pollen tube was obstructed and pollen tube collapsed, reducing further growth to the micropyle. Hodnett *et al.* (2005) indicated that adverse pistil-pollen interactions that include the pollen tube growth inhibition in wide crosses, which mightbe viewed as consequence of inharmonious genetic interactions due to genetic divergence of the species involved.

In the present study, apart from the slow rate of pollen tube growth several other types of aberrations were observed in *V. mungo*×*V. umbellata* cross. Alien pollen tubes (*V. umbellata*) typically had shown a reverse direction of growth path towards the apex of stigma (Plate 1j). Such irregulari- ties in pollen tube development were furnished in pistils of cultivated sorghum with the pollen tube of *Sorghum* *intrans* and *S. interjectum* wide crosses (Hodnett *et al.* 2005) and also in *Gossypium* spp. (Gunesekaran 1997).

Another common aberration of alien pollen tube growth in cultivated *Vigna* pistils observed in this study was the lateral expanded or swollen pollen tubes in stylar tissue (Plate 1k). This phenomenon has been found in other species as well. The cross between wheat (*Triticum aestivum*) and rye (*Secale cereale*), swollen rye pollen tubes has been noticed in wheat pistils (Jalani and Moss 1980). Wild species of Sesame (*Sesamum laciniatum*) swollen tube and a twisted growth pattern was found in cultivated Sesame (*Sesamum indicum*) style (Ganesh Ram *et al.* 2006). Al- though some degree of abnormalities observed in the present study namely slow rate of pollen growth and structural aberrations, the least number of pollen tubes were reached the embryo sac and fertilized one or two ovules. It was evidenced by the presence of few seeds in the crossed pod. Low seed set per pod was observed in crosses of different *Vigna* species (Umamaheshwari 2002).

It was suggested to use techniques such as bud pollination, application of growth hormones, *invitro* fertilization and protoplast fusion (Chen *et al.* 1989) to promote pollen germination, and pollen tube penetration in stylar and ovular tissues to effect fertilization in the wide crosses with pronounced pre-fertilization barriers. Hence, the use of one or a combination of these techniques could be utilized for the production of inter-specific hybrids and introgression of MYMV and bruchids resistance genes from the *V. umbellata* to the cultivated *V. radiata* and *V. mungo*.

Embryos have been recovered from interspecific hybrids in many species, and success has been achieved also with intergeneric hybrids of barley and rye, and of wheat and rye (Taji *et al*. 2001). As the endosperm usually fails to develop in seeds, embryos must be transferred to an artificial culture medium to allow them to grow under optimum culture conditions (Sidhu 2006). This procedure is referred to as embryo rescue. The most important aspect of the embryo rescue technique is the selection of regeneration medium necessary to sustain the continued growth of the embryo (Chawla 2002). Media can be supplemented with vitamins, growth regulators, and with natural extracts to ensure optimum conditions for embryo development.

Angelika Noga *et al*., 2016 reported Seven hundred and twenty oat panicles (17,904 florets) from 21 oat genotypes were emasculated and pollinated with maize pollen. A total of 700 haploid embryos were obtained from all genotypes, of which 133 germinated. The number of haploid embryos obtained from each genotype ranged from 5 to 83 and average embryo formation per genotype was 33.33. An average of 6.33 embryos per genotype germinated and half were acclimated to natural conditions.

Chromosome doubling

Interspecific F1-hybrids may display sterility owing to lack of chromosome pairing during meiosis. This sterility hampers further breeding. Somatic (mitotic) chromosome doubling may induce homologous pairing of chromosomes and restores fertility. Doubling the chromosome in this hybrids between mungbean (Vigna radiata) and ricebean (Vigna umbellata).

REFERANCE

[1] Ali Q, Ashraf M, Athar HR. 2007. Exogenously applied proline at different growth.Vandeleur R, Niemietz C, Tilbrook J, Tyerman SD. (2005).Roles of aquaporins in root response to irrigation. Plant and Soil 274: 141–161.

[2] Angelika Noga & Edyta Skrzypek & Marzena Warchoł1 & Ilona Czyczyło-Mysza & Kinga Dziurka & Izabela Marcińska & Katarzyna Juzoń & Tomasz Warzecha & Agnieszka Sutkowska & Zygmunt Nita3 & Krystyna Werwińska.(2016). Conversion of oat (Avena sativa L.) haploid embryos into plants in relation to embryo developmental stage and regeneration media. In Vitro Cell.Dev.Biol.—Plant 52:590–597.

[3] Bhanu, A.N., M.N.Singh and K.Srivastava. 2018. Crossability studies of interspecific hybridization among Vigna species. Biomed. J. Sci.& Tech..Res. 2(5): 2885-2891.

[4] Bharathi, A., K.S.V.Selvaraj,P.Veerabadhiran and B.S.Laksh. 2006. Crossability barriers in mungbean (Vigna radiata (L.) Wilczek): with its wild relatives. Indian J..Crop Sci. 1: 120-124.

[5] Bassiri', f. Ahmad' and a. E. Slinkard'(1986). Pollen grain germination and pollen tube growth following in vivo and in vitro self and interspecificn pollinations in annual cicer species. Euphytica 36 (1987) 667-675

[6] Basavaraja T., Niranjana Murthy, L. Vijay Kumar, K. Mallikarjun. 2018.Studies on cross compatibility in interspecific crosses of Vigna radiata × Vigna umbellata species.Legume Research - An International Journal DOI:10.18805/LR-3974

[7] Bolling, M., Sander, D. A. and Matlock, R. S. 1961. Mungbean hybridization technique. Agron J. 53: 54–55.

[8] Chaisan, T., P.Somta, P.Srinives, S.Chanprame, R.Kaveeta, S.Dumrongkittikule. 2013. Development of tetraploid plants from an interspecific hybrid between mungbean (Vigna radiata) and ricebean (Vigna umbellata). J..Crop Sci. and Biotech. 16: 45-51.

[9] Chen G, Komatsuda T, Ma JF et al (2011) A functional cutin matrix is required for plant protection against water loss. Plant Signal Behav 6:1297–1299. doi:10.4161/psb.6.9.17507.

[10] Chen H, Chen X, Chen D et al (2015) A comparison of the low temperature transcriptomes of two tomato genotypes that differ in freezing tolerance: Solanum lycopersicum and Solanum habrochaites. BMC Plant Biol 15:132. doi:10.1186/s12870-015-0521-6.

[11] Dresselhaus, T., A. Lausser and M.L. Marton (2011) Using maize as a model to study pollen tube growth and guidance, crossincompatibility and sperm delivery in grasses. Ann. Bot. 108: 727–737.

[12] Fujii, S. and K. Toriyama (2008) Genome barriers between nuclei and mitochondria exemplified by cytoplasmic male sterility. Plant Cell Physiol. 49: 1484–1494.

[13] Ganesh Ram, S., Sundaravelpandian, K., Kumar, M., Vinod. K. K., Kannan Bapu, J. R. and Raveendran, T. S. 2006. Pollen- pistil interaction in the inter-specific crosses of Sesamum sp. Euphytica 152: 379–385.

[14] Gopinathan, M. C.; Babu, C. R.; Shivanna, K. R. (1986) *Interspecific hybridization between rice bean (Vigna umbellata) and its wild relative (V. minima): fertility-sterility relationships* Euphytica, 35 (3). pp. 1017-1022. ISSN 0014-2336

[15] Gunasekaran, M. 1997. Studies on palynological features as related to crossability barriers in some diploid and tetraploid species of genus Gossypium. Ph.D Thesis, Tamil Nadu Agricultural University, Coimbatore, India.

[16] Hodnett, G. L., Burson, B. L., Rooney, W. L., Dillon, S. L. and Price H. J. 2005. Pollen-pistil interactions result in reproductive isolation between Sorghum bicolor and divergent Sorghum species. Crop. Sci. 45: 1403–1409.

[17] Jalani, B. S. and Moss, J. P. 1980. The site of action of the crossability genes (Kr1, Kr2) between Triticum and Secale. I. Pollen germination, pollen tube growth, and the number of pollen tubes. Euphytica 29: 571–579.

[18] Juanzi WuYuan QinJie Zhao (2008). Pollen tube growth is affected by exogenous hormones and correlated with hormone changes in styles in Torenia fournieri L. Volume 55, Issue 2, pp 137–148

[19] Innan, H. and F. Kondrashov (2010) The evolution of gene duplications: classifying and distinguishing between models. Nat. Rev. Genet. 11: 97–108.

[20] Kinoshita, T. (2007) Reproductive barrier and genomic imprinting in the endosperm of flowering plants. Genes Genet. Syst. 82: 177–186.

[21] Lavania, U.C., S. Srivastava, S. Lavania, S. Basu, N.K. Misra and Y. Mukai (2012) Autopolyploidy differentially influences body size in plants, but facilitates enhanced accumulation of secondary metabolites, causing increased cytosine methylation. Plant J. 71: 539– 549.

[22] Li-Jia Qu Ling Li Zijun Lan Thomas Dresselhaus (2015). Peptide signalling during the pollen tube journey and double fertilization J Exp Bot 66 (17): 5139-5150.

[23] Lord, E. M. and Russell, S. D. 2002. The mechanism of pollination and fertilization in plants. Ann. Rev. Cell Dev. Biol. 18:81–105.

[24] Lowry, D.B., J.L. Modliszewski, K.M. Wright, C.A. Wu and J.H. Willis (2008) Review. The strength and genetic basis of reproductive isolating barriers in flowering plants. Philos. Trans. R. Soc. Lond., B, Biol. Sci. 363: 3009–3021.

[25] Manickam, S. 1996. Fertilization barriers in inter-generic crosses involving maize as the female parent. Ph.D Thesis. Indian Agricultural Research Institute, New Delhi.

[26] Monika, K., Singh, P. and Saharan, R. P. 2005. Unilateral incompatibility barriers between Vigna

radiata L. Wilczek and Vigna umbellata L. Roxb. J. Cytol. Genet. 6: 171–174.

[27] Pandiyan, M., N.Senthil, N.Ramamoorthi,A.R.Muthiah, N.Tomooka, V.Duncan and T.Jayaraj. 2010. Inter-specific hybridization of Vigna radiata x 13 wild Vigna species for developing MYMV donar. Elec. J. Pl. Breed.1: 600-610.

[28] Pandiyan, M.,N.Senthil, R.Suresh, N.Chakravarthy, D.Packiraj and S.Jagadeesh. 2012. Inter-specific hybridization of Vigna radiata x V. trilobata. Wudpecker J. Agri. Res.1: 233-234.

[29] Prem Kumar. N., M.Pandiyan and R. Veerabadran. 2007.Prefertilization barriers in Vigna radiata x Vigna umbellataPLANT ARCHIVES 7(1):377-380

[30] Prithviraj S. K. and Niranjana Murthy .2018.Interspecific Hybridization between Mungbean (Vigna radiata) and Rice Bean(Vigna umbellata) for Crossability and Related Parameters .Mysore J. Agric. Sci., 52 (2) : 316-322, 2018

[31] Sarker, R. H., Samad, M. A., Seraj, Z. I., Hoque, M. I. and Islam, A. S. 1983. Pollen tube growth in crosses between Porteresia coarctata and Oryza sativa. Euphytica 69: 129–134.

[32] Sidhu PK, Howes NK, Aung T, Zwer PK, Davies PA (2006) Factors affecting oat haploid production following oat×maize hybridization. Plant Breed 125:243–247.

[33] Shayla Bindra, R.K. Mittal, V.K. Sood and Chaudhary. H.K. 2020. Alien Introgression Studies. Involving Vigna mungo x Vigna umbellata Hybridization. Int.J.Curr.Microbiol. App.Sci. 9(02): 268-276. doi: https://doi.org/10.20546/ijcmas.2020.902.034

[34] Taji A, Kumar P, Lakshmanan P (2001) In vitro plant breeding. Haworth Press, Inc., New York, London, Oxford, p. 156.

[35] Thiyagu, K.,P.Jayamani and N.Nadarajan.2008. Pollen pistil interaction in inter-specific crosses of Vigna species.Cytologia 73:251-257.

[36] Umamaheshwari, D. 2002. Wide hybridization in the genus vigna. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, India.

[37] Watanasit, A. and Pichitporn, S. 1996. Improvement of mungbean for resistance to bruchids. In: Srinives P., Kitbamroong C.and Miyazaki S. (eds.) Mungbean germplasm: collection, evaluation and utilization for breeding program. Japan International Research Center for Agricultural Sciences, Tsukuba, Japan, pp. 67–71

[38] Yamagishi, H. and S.R. Bhat (2014) Cytoplasmic male sterility in Brassicaceae crops. Breed. Sci. 64: 38–47.