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Cytological Irregularities Occurs in F1s of Vigna Radiata X Vigna Umbellata Cross Combination

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Abstract: Cytological irregularities occur when ever breeders attempted wide hybridization among thecrops plants. The plant breeders having knowledge on their crops to be improved the traits. This study focusses on cytological irregularities in F1s of Vigna radiata x Vigna umbellataof interspecific hybridization.There is a cross compatibility between these twospecies evenhaddistinct differencein morphology. Even though easy cross compatibility between these two dissimilar species for crossing and formed fertile seeds to germinate as F1s plants. The germinated F1 plants produced complete sterile pollen grains, thenon fertilepollen grainsreached on stigma surfaceof same F1 plants and therewas nopollen germination followed by no pod formation. Byselfingof same flowers, pollen grains of different flowers of same cross were collected and dusted on the same stigmasof same cross there was no pollen germinationand pod formation due to meiotic distraction which leads to male sterility. The same F1s flowers were backcrossed with both parentsalso not pollen germinated and no pod formed due to megaspore disintegrated leads to female sterility. True F1 plants were produced highest number of flowers and nopod formationdue to male and female sterility. Detailed meiotic studies were carried out for male and female organs ofF1s plants and the foundreasons.Detailed study was examined for microsporegenesisof PMCs , the several meiotic irregularities namely chromatins irregulaties , univalent, bivalent, quadrivalent, dyad, triad, tetrad, pentad, hexad, octad and multiloids and alsofound frequent nonseparation of tetrad. The parents exhibited the same normal chromosome number 2n = 22, normal ovule , normal pollen grain fertility, pollen germination, perfect separation of tetrad and F1s showed high frequency of total meiotic chromosomal aberrations. Various types of meitotic abnormalities such ascytomixis, lagging chromosomes, chromosomal

bridges, and chromosome stickiness have been observed in all F1 plants. The cytological findings revealed that F1s had both male and female sterility occurred in F1s of Vigna radiata x Vigna umbellata crosses. Studying the cytological behavior of this cross combination will give ideas for young Vigna breederswho are doing future interspecific hybridization of Vigna species related to irregularities happened in Vignaspecies andindebthresearch may be intensified for future Vignabreeding programme.

Keywords: Vigna species- Cytological irregularities– Pollen sterility- Microspore and Megaspore irregularity- Pollen stigma interaction- Ovule obnormalities-DNA F1 confirmation.

Introduction

Cytological irregularities occur when ever breeders attempted wide hybridization among crops plants. The plant breeders having knowledge on their crops to be improved the traits. The breeders are struggling to get targeted traits into desired genotypes when ever goes for wide hybridization to develop resistant genotypes in improving ruling varieties. The difficulties faced in attempting cross between two different species of same genera or other genera. The breeders getting cytogenetic problems due to meiotic irregularities are involved in F1s generation. The crossed pods were harvested and seeds were extracted from crossed pods (1, 2). Objective of the study was to step wise know the reasons for male and female sterility and overcome the problems (3)

Materials and Methods

Parents of mungbean and rice bean were raised in crossing block at Agricultural Research Station, Virinjipuram, Vellore during rabi 2015-2016. Crossing was made between these two species. Crossed pods were harvested and extracted the seeds from crossed pods as considered as F_0 seeds.

F_0 seeds of Vigna radiata x Vigna umbellata crossed seeds were sown at middle both sides of the crossed seeds , the female and male parents seeds sown during Kharif -2016 at Agricultural Research Station, Virinjipuram, Vellore, the analysis were started in the flowering stages. DNA analysis were taken in the recombinant plants of crosses to find true hybridity.

The DNA extraction procedure was adopted as protocol DNA extracted from good leaves and DNA from old or damaged leaves were extracted using machine, Qiagen E21 roboEZ1

DNA was extracted by following method. DNA extracted from good leaves and DNA from old or damaged leaves were extracted using machine, Qiagen E21 roboEZ1

Protocol for isolation of genomic DNA from bean leaves

Leaf sample in 1.1ml tube including 1 tungsten bead (stored at –80C on 96 rack)
↓crush leave using Qiagen MM300 (25Hz/30sec, 2 directions)
↓set the 96 rack
↓add 400µl of **Digestion Buffer Working solution** [1] and put cap
↓mix well
↓incubate at 55℃ for 1-1.5 hr
↓add 350µl of **Phenol/chloroform/isoamylalcohol**[2], and invert 10 times
↓9000 rpm for 5 min x 2 directions
↓transfer 300µl of supernatant to new 1.1ml tube
↓add 300µl of **chloroform/isoamylalcohol**[2], and invert 10 times
↓9000 rpm for 5 min x 2 directions
↓transfer 250µl of supernatant to new 1.1ml tube
↓add 250µl of **isopropanol**, and invert 10 times (DNA fiber will appear)
↓9000 rpm for 5 min
↓reverse tube stand and 9000 rpm for 5 min
↓Remove supernatant using pipette
↓add 300µl of **EtOH (70%)**, and vortex (wash tube wall by the ethanol)
↓incubate for 5 min (elute salt from DNA to the ethanol)
↓9000 rpm for 5 min
↓Remove supernatant using pipette (200µl)
↓9000 rpm for 1 min
↓Remove supernatant using pipette (200µl) as completely as possible
↓add 200µl of **TE-RNase solution**[3], and vortex
↓spin down
↓incubate at 37℃ for 30 min
Store at -20℃

[1] Digestion Buffer Working solution for 96 samples (prepare before use)

1.3 ml	Proteinase K solution (Qiagen)
45ml	Extraction Buffer
900ul	2-mercaptoethanol

[2] Phenol/chloroform/isoamylalcohol(25:24:1) for 96 samples

24 ml	chloroform
1 ml	isoamylalcohol

25 ml TE saturated phenol

[3] TE-RNase solution

10 ml TE (10mM, 0.1mM)

200µl RNase (10mg/ml)

1% Gel preparation (for check concentration)

- Add 2.0 g agarose to 200 ml of 1xTAE
  ↓
- Boil in microven and the solution should be clear and transparent
  ↓
- Prepare gel tray and comb, mold
  ↓
- Pour gels in gel mold and leave for 1 hours at room temperature (gel must be solidified)
  ↓ while waiting for the gel to solidify prepare the samples to be loaded in the gel
- Mix the DNA samples and dye
  ↓

  After gel solidifies, pour small amount of 1xTAE to the gel and then gently remove the comb

  ↓

  Load the sample to the comb

Electrophoresis

- Set electrophoresis unit
  ↓
- Keep 1 x TAE buffer in tank
  ↓
- Keep gel in tank buffer (gel should be sub merged in buffer)
  ↓

- Prepare sample to load
  ↓
- Run sample 20 min, stain with EtBr for 1 hour.
  ↓
- Take picture and compare intensity of sample signal with λDNA standard

Polymerase Chain Reaction (PCR)

Reagents	**20 µl in 1 sample**
10xPCR buffer	2 µl
2.5 mM dNTP	1,6 µl
10µM AT1 primer (Farward)	2 µl
RB primer (Riverse)	2 µl
MQ water	10,2 µl
Ex Taq	0,2 µl
Template DNA	2 µl

PCR set up:

Temperature	**Time**	**№ of cycle**
94^0 C	**30 sec**	**35 cycle**
94^0 C	**30 sec**	
52^0 C	**30 sec**	
72^0 C	**30 sec**	
4^0 C	**Hold**	

Gel Electrophoresis

↓

Prepare 1, 5 % agarose gel.

↓

Mix the PCR sample with Dye

↓

Load Hind III marker to the comb

↓

Run sample 20 min, stain with EtBr for 1 hour.

↓

Take picture

In all F1 plants expressed the hybrid sterility which means suppression of reproductive capacity of F1s. Assesed all the F1 plants for reproductive sterility.

$$\text{Hybrid sterility} = \frac{\text{No. of plants sterile}}{\text{No. of plants assessed}} \times 100$$

The hybrid leathality also assessed, numbers of plants survived over germinated seeds were taken to assess the lethality of F_1 hybrids.

$$\text{Hybrid lethality} = \frac{\text{No. of plants died}}{\text{No. of seeds germinated}} \times 100$$

The pollen fertility analysis was carried out in the parents and their hybrids by an acetocarmine staining technique

$$\text{Pollen fertility} = \frac{\text{No. of viable pollen}}{\text{Total no. of pollen observed}} \times 100$$

Meiotic chromosomes of pollen mother cells were studied by using Rapid Squash technique (4). The small flower buds were collected from experimental plot during morning time between 6.00 A.M. and 7.30 A.M in Carnoy's fixative solution having 3:1 ratio of absolute ethyl alcohol: acetic acid for carrying them to laboratory. The anthers from appropriate size of buds were smeared in Lacto propionic orcein and visualized in photographic compound microscope. The metaphase plate, anaphase and diakinesis stages were observed in the F1s. The Pollen mother cells were observed for the study of meiosis in F1s

For preparing slides, the anthers were squashed in 2 per cent aceto carmine and the slides were slightly warmed and observed under a light microscope for pollen fertility.

The chromosome association at meiosis was studied for the hybrids. Cells at diakinesis, metaphase and anaphase were examined to obtain the frequencies of univalent, bivalent, quadrivalent and other valents. Twenty five PMCs were observed for estimating the frequencies of chromosomal abnormalities (cytomixis, univalent formation, chromosome stickiness, precocious separation, unequal separation, laggard, dyads, triads, tetrad, pentad, hexad, octod and multiloids). Photomicrograhs were taken for various abnormalities observed in the hybrids.

Results

The results of F1s plant showed hybrid sterility and leathality (Table 2). All the F1 hybrid plants showed 100 percent reproductive sterility and 100 percent pollen sterility was observed. Recorded some of the plants were dead by hybrid lethality. The live plants were seems to be normal, all reproductive stages like flower initiation, flower growth and flower opening and anthesis were normal. The F1 plants were produced more number of flowers 2500 to 4000 per plant. F1 plants were self pollinated with same flower of pollen grains and also other flowers of same crosses, there was no pollen germination and podformationwhich showed male sterility and back crossed with parents even then there was no pollen germination on stigmatic and no pod sets which showed female sterility. The keen observation was made visibly, sterility for both male and female organs in the F1 plants were recorded. As a first step the pollen was tested for fertility by acetocarmine methods, all the pollens were not stained so that pollen was observed as complete sterile pollen which shows male sterility where as in the parents normal fertility observed (Fig 5) Table 3. For testing of female organs dissect the ovule by using stains which is also not staining its showed female sterility where as normal in parents. For finding more reason, the genetic studies were taken place at meiosis level for both male a female organs sterility. There was no pod set due to meiotic irregularities particularly non separation of tetrad formale sterilityand megaspore formation followed by

degeneration of megaspore where as in parents was normal. Apart from these meiotic irregularities in the PMCs, univalent formation, reduced number of bivalents, quadrivalents, cytomixis, chromosome stickiness, precocious separation, unequal separation, laggard, dyads, triads, tetrad, pentad, hexad, octod and multiloidwere observed (Table .1).

In meiosis study for male sterility examined the PMCs aftertetrad formation there is high frequency of non separation of single haploid cells which leads to male sterility where as in parents shown normal separation which leads to productionof fertile male gametes (Fig 4).The selected individual chromosomes meiotic studies carried out in Pollen Mother Cells (PMCs) of F1s of Vigna radiata x Vigna umbellata cross combination. Chromosome associations were noted during diakinesis, metaphase and anaphase of F1 plants. Meiotic studies revealed the details of chromosome associations which varied from cell to cell both between and within different F1 plants. Three different types of associations are present in all the chromosomes studied. (Table.1)

PMCs 25 cells involving 330 chromosomes showed that maximum i.e. 52% cells were associated as univalents, 37.57 % bivalents and 9.69 % quadrivalents. The cytogenetic analysis through meiotic studies in PMCs was carried out. The result was presented in Table 1. Both species has 2n = 22 chromosomes and meiosis was normal with regular formation of 11 bivalents. In $F_1$of their cross, abnormalities were observed in all the plants. Out of 25 PMCs studied at Anaphase I, only one cell revealed 11 bivalents. The occurrence of abnormal association namely univalent and quadrivalent was frequently observed. The number of univalent varied from 0 to 14 while number of quadrivalent ranged from 0 to 5. The average chromosome association per cell was IV (1.28) + II (4.96) + I (6.96). Precautious separation of chromosomes and formation of anaphase bridges was commonly observed in many PMCs.For female sterility abnormality in the development of megaspore particularly degeneration of embryo sac which leads to female sterility where as parents are normal production of embryo sac (Fig 4).

Chrmosomal abbrerations of various stages were observed in all the PMCs at the level of cytomixis 0.1 , chromosomal stickiness ranged from 0.1 to 0.3,Precocious separation ranged from 0.1 to 0.2, Un equal separation observed in PMCs 2 to 4, dyad 1 to 3, triad 1 to 6, tetrad 1 to 4, pentad 1 to 3, hexad 1 to 4, multiloid 1 to 2 and , polyad 1 to 2.Cytomixis is genetic materials transferred from one cell to another cell by alteration of genetic variation by different dissimilar parents involved for crossing some timeshappened multiple cytoplasmic connections at prophase in PMCs of F1s. Cytomixiswere observed in all the PMCs at the level of cytomixis 0.1. The chromosome stickiness is clumps of chromatin

resulting in sterility at high frequency in the sterile F1s. The chromosome stickiness delays the normal separation of bivalents and lead to the formation of laggards (chromosomes did not overlap along the long axis of the spindle with any of the properly segregating chromosome) observed chromosomal stickiness ranged from 0.1 to 0.3 In the current study, the precocious separation may be the maximum frequency of unequal separation has been noticed. Precocious separation ranged from 0.1 to 0.2, non-synchronous disjunction due to chiasmata interlocking, lack of coordination between spindle and chromosomes.

Un equal separation observed in PMCs 2 to 4.The chromatin bridge formation is a bridge formed between the separating groups of anaphase chromosomes because the two centromeres of centric chromosome are being drawn to opposite poles. In the present study, the occurrence of chromosome bridges at anaphase-I could be due to stickiness of chromosomes at metaphase I, which restrict their movement towards opposite poles.Delayed terminalisation, formed abnormal spindles and chromosomal movement failure and the formation of increased frequency of laggards. PMCs with disturbed polarity by irregular spindle activity due to abnormal introgression two dissimilar genotypes cross combinations.Abnormal microsporogenesis or irregular microsporogenesis due to aberrant meiotic results in abnormal sporads such as dyads, triads, pentad, octad, multiloids and polyads.

Hybrid sterility

The hybrid sterility observed in all the 15 plants recovered till maturity, hybrid sterility percentage was recorded 100 percent.

Hybrid Leathality

Out of 50 seeds sown 35 seeds were germinated and only 15 plants survived at maturity. The percentage of hybridleathality for hybrid was 12.85.

Pollen fertility

The F1s plants of this cross combination produced highest number of flowers and anthers produced more number of sterile pollen progressively decline. The percentage of parents pollen fertility was observed for 98.50 and 89.75 in Vigna radiata and Vigna umbellata respectively. Inthe hybrid, the pollen fertility percentage was 0.0 percent.

Confirmation of the true F1 through marker

Finding the conformation of true recombinant plants in Vigna radiata x Vigna umbellata cross combinations tested with 40 primers

The primers have given valuable evidence of the umbellata gene present in the hybrid derivative (Fig.6). This line is highly resistant to mungbean yellow mosaic virus throughout the generation. like umbellata If test all the lines in this population by more number of markers there is a possibility to tag the resistance genes which will be help to plant breeders. The primers G263 primer G 284 and G 285 used for detecting polymorphism

Discussion

Crop species have been improved by hybridization foruseful traits and accumulating desirable genes from genetic resources. "Hybridization breeding" introduces valuable traits like biotic or abiotic tolerance into existing cultivarsfrom breeding stocks or wild progenitors by intraspecific or interspecific crossing. As genetic resources are limited in intraspecificand interspecific crossing is expected to contribute to further development of plant breeding programs.

In the interspecific crossing, irregular genetic distractive mechanisms are highly interrupted to stop the gene flow with others. Naturally pollination is restricted by their natural habitat (geographic isolation), structure of flowers (i.e., shape or color), flowering time or pollen mediator which are involved in pre zygotic barriers (5). In this study F1s has the reproductive barrier mechanism related to meiotic irregularities and degeneration of female part.

Present study having reproductive distructive problem of no pollen germination, no pollen tube germination inthe F1s of Vigna radiata x Vigna umbellata crosses.The pollen and pollen tube germination inhibited by the Inter-specific incompatibility is involved and the pollen from other species pollinate the stigma which inhibit the pollen germination followed by pollen tube growth in pistil portion (6). The endosperm abnormality isformed dependson the cross combination of different species usually happened in inerspecific hybridization andalso ploidy levels of parental species (7).

(8) Results revealed that Interspesific F1 hybrids between S. aethiopicum Acc.8971 and S. melongena var. DLP and Acc. 3305 were produced. There are no recovered crosses were observed and pre-fertilization barriers causing hybrid sterility and breakdown. Fluorescence microscopy was used to determine pre-fertilization barriers. Pre-fertilization barrier in the form of inhibited pollen germination and tube growth was noted.

Varying degrees of success in interspecific hybridization and also meiotic irregularities reported by various workers like (9, 10, 11,12,13) for interspecific hybridization among four species of the genus Vigna savi (14) owing to reproductive obstructions between the species involved in interspecific hybridization (14), (15, 16 , 17, 11 ,14, 18).

Our result is agreed with previous workers for pre and posts fertilization that reproductive barriers as hybrid weakness andhybrid sterility caused by epistatic interactions between nuclear genes or between the nuclear genome and the maternally derived mitochondrial genome, termed cytoplasmic male sterility (CMS), remains as a post-zygotic barrier. However, male sterility is sometimes useful for breeding, and CMS is successfully applied to harvest the F1 hybrid seeds (19). Several genes involved in hybrid sterility or CMS in rice have been isolated by a genetic approach (20). Overcoming reproductive barriers expands the possibility of hybridization breeding, and understanding the molecular mechanism of reproductive barriers is becoming more important.

This kind of problems arises in interspecific hybrids of such polyploidy crops viz., maize, cotton, wheat, canola, tobacco, potato, and strawberry, indicating that polyploidizationby (21) and (22) Allopolyploid species in heterozygosity (10).

The same results were obtained for Inter-specific hybridization between mungbean (V. radiata (L.) Wilczek) and Adzukibean (V. angularis (wild) by (23) and (16) for induced genetic variability in mungbean through inter-specific hybridization. (10) revealed the Inter-specific hybridization between V. radiata (L.) Wilczek and V. glabrescens.and incrossability barriers in some diploid and tetraploid species of genus Gossypium by (24, 25) found the fertilization barriers in inter-generic crosses involving maize as the female parent. (26) found the same results in mungbean incompatibility barriers between Vigna radiata L. Wilczek and Vigna umbellata. (27). Inheritance of seed resistance to bruchids in cultivated mungbean.(28) has agreed for incompatibility in direct and reciprocal cross between S. indicum L. and S. alatum.

(29), The 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.(30) higher pod harvest was seen in crosses with V. unguiculata as female parent. GA treatment enhanced pollen germination and pollen tube growth, and by partially overcoming embryo abortion for 10 to 12 d, immature embryos were successfully rescued for in vitro production of hybrid plantlets

The Pollen mother cells observed for the study of meiosis in parents showed all the stages of meiosis i.e Leptotene, Zygotene, Pachytene, Diplotene, Diakinesis, Metaphase I, Anaphase I, Telophase I, Metaphase II, Anaphase II, Telophase II and tetrads were clearly observed. The Chromosome pairing behaviour and Chromosomal association at diakinesis stage in parents like mungbean and rice bean revealedmaxmimum paring as bivalents. Since in vigna radiata 2n=22,

therefore, 11 bivalents were observed in maximum number of cells i.e. 80% cells (Table 1). At Diakinesis stage the other chromosomal associations observed were 10 bivalents + 2 univalents, 9 bivalents + 4 univalents both in 8% cells. Also, it was interesting to observe 12 bivalents in 4% cells i.e. showing 2n=24 (Fig 1) which has also been reported earlier.In case of Lablab, at Metaphase stage also some meiotic anomalies were observed in the pollen mother cells i.e. single chromosome bridge and laggards during Anaphase stage

Hybrid sterility

Interspecific hybrid sterility is a common form of reproductive isolation in rice (Oryzasativa L.), (1, 2). Similar finding agreed with various authors, (23) sterility observed in Interspecific hybridization between mungbean Vigna radiata L. Wilczek and adzuki bean Vigna angularis (31). Stwrility in interspecific hybridization between Vigna unguiculata and Vigna vexillata (32). Chromosome namalis in Cultivated Plants (33) Chromosome behaviour and structural hybridity in the tradescantiae. (34). Interspecific hybridization between rice bean (Vigna umbellata) and its wild relative (V. minima), (35). Sterility observed in interspecific hybridizations in Pennisetum species. (36). Sterility observed in interspecific hybrids (37). Sterility observed in interspecific hybridization studies in Vigna radiata L. Wilczek and Vignaumbellata

Hybrid leathality

Hybrids between closely related species are sometimes partially fertile. Gene exchange may nevertheless be inhibited because the offspring are poorly viable or sterile. Hybrids between the cotton species Gossypiumbarbadense, G. hirsutum, and G. tomentosumappear vigorous and fertile, but their progenies die in seed or early in development, or they develop into sparse, weak plants.(24) and in Vigna species interspecific hybridization by (1,2)

(29). This study was got successful pod set in 12 crosses out of 25 interspecific crosses of Vigna radiata and Vigna umbellata. further noticed are strong pre-fertilization as well as post fertilization barriers were present in the cross between V. radiata and V. umbellata , while, growth and lethality of interspecific hybrid seedlings were influenced by the genotypes of both the parental species.

(38, and 3) studies pointed out Vigna radiata x Vigna umbellata.crossability status and further F1s non survivality due to pre and post fertilization barrier and leathality also observed in F1s

Pollen fertility

The F1s plants of this cross combination produced highest flowers and anthers produced more number of sterile pollen progressively decline. The same kind of pollen problem of results were obtained in various crops such as garden beans by (39) lentils (40, 41), horse gram (42) and pigeon pea (43).

The total reduced pollen fertility in the F1 plants due to chromosomal aberrations that also occurred at higher frequencies by cytogenetical distraction. The earlier studies also supported this view that reduced pollen sterility occurs by induced mutagens which leads chromosomal anomalies and hence genetic alterations resulting in aberrant pollen grains (44), (45) and (46) and in Vigna species interspecific hybridization by (1,2)

(47) Pollenbehaviour of different varieties and mutants of variety SB-42. Longestpollen tube (98.37μm) and highest pollen germination (56.61%) obtained from Mutant 24 and mutant 11respectively hence can be used as a male and female parent in hybridization respectively and pollen sterility by (48)

Cytological aberrations

Under normal conditions cell are acted meiotically given cytological stages of the cell cycle in germ cells. Present study parental union of gamets are normal, in the F1s plants showed abnormalities of crossing over and linkage which leads alterations in any of these genes are deleterious and result in severe meiotic abnormalities that may impact the species non viability and overall un reproductive capacity (49, 50, 51, 52) Meiotic abnormalities always lead to the formation of chromosomally imbalanced gametes, which ultimately affect the pollen by several workers (53, 54).

Present investigation, many meiotic abnormalities such as cytomixis, univalent formation, chromosome stickiness, precocious separation, unequal separation, laggard, dyads, triads, tetrad, pentad, hexad, octadand multiloidshave been observed in the course of meiosis in most of the analyzed PMCs. Frequency of meiotic abnormalities was formed by mutagenic treatments of many findings (55,56,57) and (58) who reported maximum meiotic abnormalities in PMCs of urdbean treated with combined mutagen treatments.

Cytomixis is genetic materials transferred from one cell to another cell by alteration of genetic variation by different dissimilar parents involved for crossing some time shappened multiple cytoplasmic connections at prophase-I/II in PMCs of F1s. Cytomixis may lead to the formation of chromosomal aborations (59).

The cytomixis has also been reported in many crop plants (54, 60, 61), (62 and 63). Earlier workers have reported that cytomixis may be induced by several

factors, such as temperature (64), the combined role of stress and genetic factors (65), and direct genetic control (66), (67).

Univalents occurred at metaphase-I that fails to pair at zygotene and remains separated at diplotene due to the lack of chiasma formation or the precocious separation of bivalents at anaphase. The same results obtained with the reports of Amin et al.(2019)that showed a maximum frequency of univalents in black cumin mutants . The absence of pairing that lead to failure of chromosome movement and disturbed normal pairing of homologous chromosomes (55), (68) and in Vigna species interspecific hybridization by (1,2).

In the present study observed the chromosome stickiness is clumps of chromatin resulting in sterility at high frequency in the sterile F1s. The chromosome stickiness delays the normal separation of bivalents and lead to the formation of laggards (chromosomes did not overlap along the long axis of the spindle with any of the properly segregating chromosome), chromatin bridges, micronuclei, and sterile pollen grains. Induced chromosome stickiness in faba bean by (55 and 69).

. The chromosome stickiness could also be induced by other factors such as soil elements (70, 71) herbicides (72), and soil chemicals (73) and (74). The cytological phenomenon where homologous chromosomes pair showed a lack of synchrony in their segregation at metaphase-I or anaphase-I is known as non-synchronous disjunction.

Non-synchronous disjunction is a pair of homologous chromosomes has failed to separate or segregate at anaphase so that both chromosome of the pairs pass to same daughter cell can arise spontaneously (75) or may be developed in interspecific crosses (3, 76) or be induced by different mutagens (77), (78, 79) In the current study, the precocious separation may be the maximum frequency of unequal separation has been noticed by (56), (3,76). Non-synchronous disjunction due to chiasmata interlocking, lack of coordination between spindle and chromosomes (80) varied rates of terminalisation (81).

(82) altered chromosome homology (83). The disturbed orientation and separation of bivalents resulted in chromatin bridges, laggards and unequal distribution of chromosomes at poles during anaphases, ultimately affecting pollen fertility (3,76).

The chromatin bridge formation is a bridge formed between the separating groups of anaphase chromosomes because the two centromeres oficentric

chromosome are being drawn to opposite poles . It is observed for the first time by Greet in 1911, (84). During cell division two centromeres in the chromosome is dragged to the opposite poles of the cell. Several authors provided explanations for the origins of bridge formation, including paracentric inversion (85), failure of chiasmata in a bivalent to terminalize (86) and interlocking of bivalent chromosomes (87) The sister chromatid attributed to bridge formation and chromatin exchange followed by delayed or non separation at subsequent stages due to interspecific or induced formation of anaphase bridges may also be attributed to the chromosome breakage followed by a reunion of broken ends (88,and 89) also believe that breakage and reunion of these chromosomes lead to the formation of dicentric chromosomes that result in the formation of single and multiple bridges. In the present study, the occurrence of chromosome bridges at anaphase-I could be due to stickiness of chromosomes at metaphase I, which restrict their movement towards opposite poles.

Delayed terminalisation, formed abnormal spindles, and chromosomal movement failure and the formation of increased frequency of laggards. The mutagenic studied results given as same results were in accordance with our findings of (79).

PMCs with disturbed polarity by irregular spindle activity due to abnormal introgression two dissimilar genotypes cross combinations (81,90, 91) with the findings of (55).

Abnormal microsporogenesis or irregular microsporogenesis due to aberrant meiotic results in abnormal sporads such as dyads, triads, multiloids and polyads. Several workers have also reported the formation of dyads, triads, and polyads in Tuberosum (92), (93) potato (94) and roses (95) The lack and absence of cytokinesis in meiosis II may be formed of dyads with two diploid cells and triads with two haploid cells and one diploid cell (96, and 97)

According to (32) chromosome numbers for these four species were first reported as 2n = 22 **by Karpechenkoin 1925**, reported 2n = 24 for black gram in 1929. All reported 2n = 22 for black gram.(98) occasionally observed precocious separation of one bivalent in meiosis of mung bean that was regular otherwise. Chromosomal aberrations due to mutagens in many cultivated species have been carried out by several workers (99,100,101,102, 103 ,104,105).The NaCl ions toxicity induced various cytological anomalies in seedling roots that adversely affect the growth of Vigna seedlings followed by reproductive distractions (106).

DNA analysis Recombination in hybrid was confirmed using these polymorphic DNA markers. The NM 2006 × Mash 88 was most successful interspecific cross. Most of true recombinants confirmed by molecular markers were from this cross combination. SSR markers were efficient indetecting genetic variability and recombination with reference to specific chromosomes and particular loci. SSR (RIS) and RAPD identified variability dispersed throughout the genome. In conclusion, DNA based marker assisted selection (MAS) efficiently confirmed the interspecific recombinants by (107). (111) and (112) used SSR markers and identified EST-SSR markers (108). (113) carried out SSR analysis of mungbean based on an SSR that can be used in marker assisted selection in mungbean. (109) and (110) also used URP, RAPD and SSR markers for genetic differentiation of Vigna species

Conclusion

To overcome this barrier problem by using more number of accessions from different species used in the conventional crossing programme and also physical methods of mutagenic agent may be used to alter the unwanted barrier for trait improvement.

Table 1. Meiotic behavior of chromatin in V. radiata x V. umbellatacross

No.of Cells	I(Univalent)	II(Bivalent)	IV(Quadrivalent)	Association range	Total number of valents in PMCs	cytomixis	Chromosomal stickiness	Precocious separation	Un equal separation	Dyad	Triad	Tetrad	pentad	Hexad	Multiloid	Polyad
PMC 1	-	11	-	11- II	11	-	-	-	-	-	-	-	-	-	-	-
PMC 2	10	2	2	10- I, 2-II, 2-IV	14	0.11	0.2	0.2	2	2	3	1	2	2	2	1
PMC 3	-	1	5	1-II, 5 -IV	6	0.11	0.1	0.1	3	2	3	3	1	1	1	1
PMC 4	4	7	1	4-I, 7-II,1-IV	12	0.11	0.2	0.2	3	2	3	2	3	3	1	1
PMC 5	10	2	2	10-I, 2-II, 2-IV	14	0.11	0.1	0.1	2	1	4	2	2	2	1	1
PMC 6	2	-	5	2-I, 5-IV	7	0.11	0.1	0.1	2	1	1	1	2	2	1	2
PMC 7	4	9	-	4-I, 9-II	13	0.11	0.21	0.21	1	1	4	4	1	1	1	1

PMC 8	10	2	2	10-I, 2-II, 2-IV	14	0.11	0.11	0.21	4	1	3	2	4	4	1	1
PMC 9	2	10	-	2-I, 10-II	12	0.11	0.11	0.1	2	1	3	3	2	2	1	1
PMC 10	4	9	-	4-I, 9-II	13	0.11	0.21	0.2	3	1	2	3	3	3	1	1
PMC 11	8	3	2	8-I, 3-II, 2-IV	13	0.11	0.11	0.1	2	1	3	2	3	3	1	1
PMC 12	10	6	-	10-I, 6-II	16	0.11	0.31	0.1	3	2	5	1	1	1	1	1
PMC 13	12	5	-	12-I,5-II	17	0.11	0.11	0.21	2	3	5	1	1	1	1	1
PMC 14	12	5	-	12-I,5-II	17	0.11	0.11		2	1	5	1	1	1	1	1
PMC 15	-	5	3	5II, 3-IV	8	0.11	0.11	0.2	2	1	2	1	1	1	1	1
PMC 16	6	4	2	6-I,4-II,2IV	12	0.11	0.11	0.1	2	2	3	1	1	3	1	1
PMC 17	12	5	-	12-I,5II	17	0.11	0.11	0.1	3	1	4	1	1	2	1	1
PMC 18	8	3	2	8-I,3-II,2-IV	13	0.11	0.11	0.21	4	2	2	1	1	2	1	2
PMC 19	12	5	-	12-I, 5-II	17	0.11	0.21	0.1	3	3	4	1	1	1	1	1
PMC 20	14	-	2	14-I, 2IV	16	0.11	0.11	0.2	4	3	6	1	1	4	1	1
PMC 21	4	9	-	4-I,9II	13	0.11	0.11	0.1	2	3	4	1	1	2	1	1
PMC 22	10	2	2	10-I,2II, 2IV	14	0.11	0.11	0.1	4	4	3	1	1	1	1	1
PMC 23	2	10	-	2-I,10-II	12	0.11	0.11	0.21	2	2	2	1	1	1	1	1
PMC 24	14	2	1	14-I, 2II, 1-IV	17	0.11	0.11	0.2	4	2	5	1	1	1	1	1

PMC 25	4	7	1	4-I,7-II, 1-IV	12	0.11	0.2	0.1	2	2	3	1	1	1	1	1
Total	174	124	32	174-I, 124-II, 32-IV	330	2.5	3.2	3.3	61	52	82	35	37	45	26	27
Average chromosome association	I (6.96)	II (4.96)	IV (1.28)	I (6.96), II (4.96), IV (1.28)	13.2	0.1	0.12	0.13	2.44	2.08	3.28	1.4	1.48	1.8	1.04	1.08

Table 2.Hybrid sterility and Hybrid leathality of V.radiata x V.umbellata cross

Parents and Crosses	No. of crossed seeds obtained	No. of seeds germinated	Hybrid lethality (%)	Hybrid sterility (%)	Germination (%)	No. of seedlings attained maturity
V. radiata	100 (parental seeds)	96	-		96.00	95.00
V. umbellata	100 (parent seeds)	92	-		92.00	90.00
V. radiata × V. umbellata	50	35	12.85	100	70.00	15.00

Table 3. Pollen fertility percentage of parents and F1 hybrids of interspecific crosses for**V.radiata x V.umbellata crosses**

Sl.No	Parents and hybrids	Pollen fertility (%)
1.	V. radiata	98.50
2.	V. umbellate	89.75
3.	V. radiata × V. umbellata	100

.

Normal Ovule observed in parents

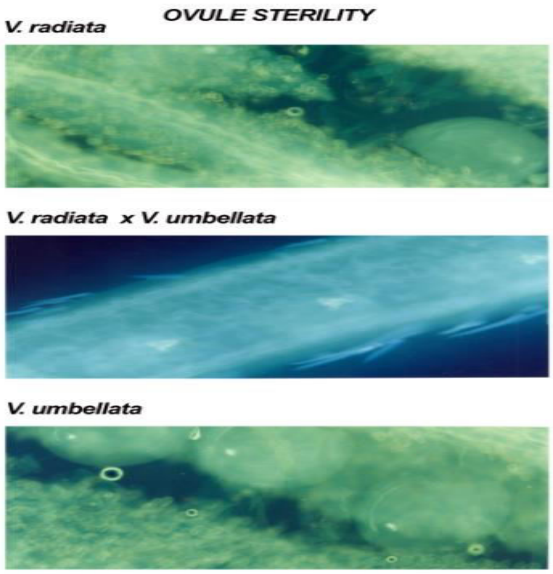


Fig .1. Parents normal ovule production in ovary of V. radiata and V. umbellata, F1s not produced normal ovules

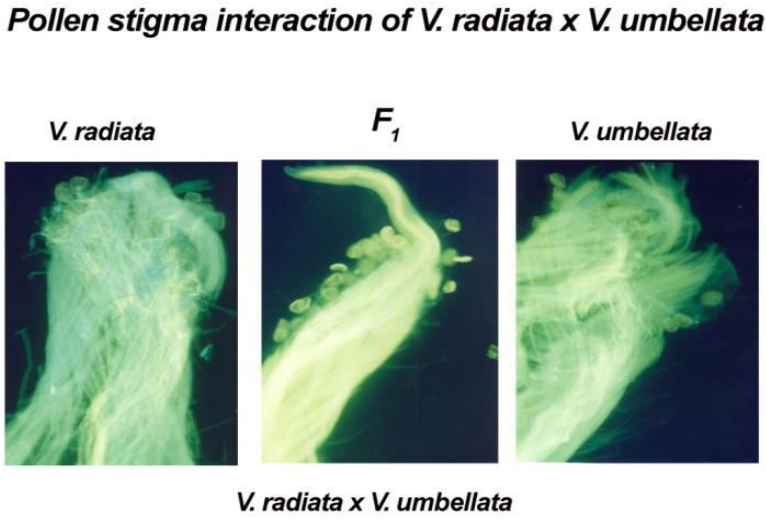


Fig .2.Stigma and pollen interaction:Normal pollen germination on stigmatic portion of parents and no germination on stigmatic portion of hybrids of Vigna radiata and Vigna umbellata crosses

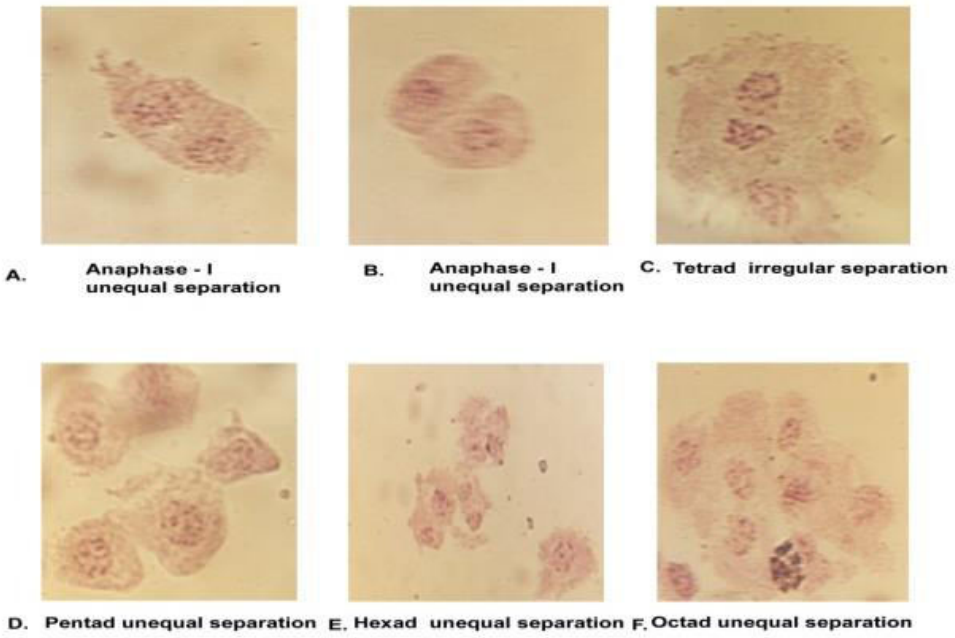


Fig 3.Meiotic abnormalities of PMC in *Vigna radiata x Vigna umbellata*

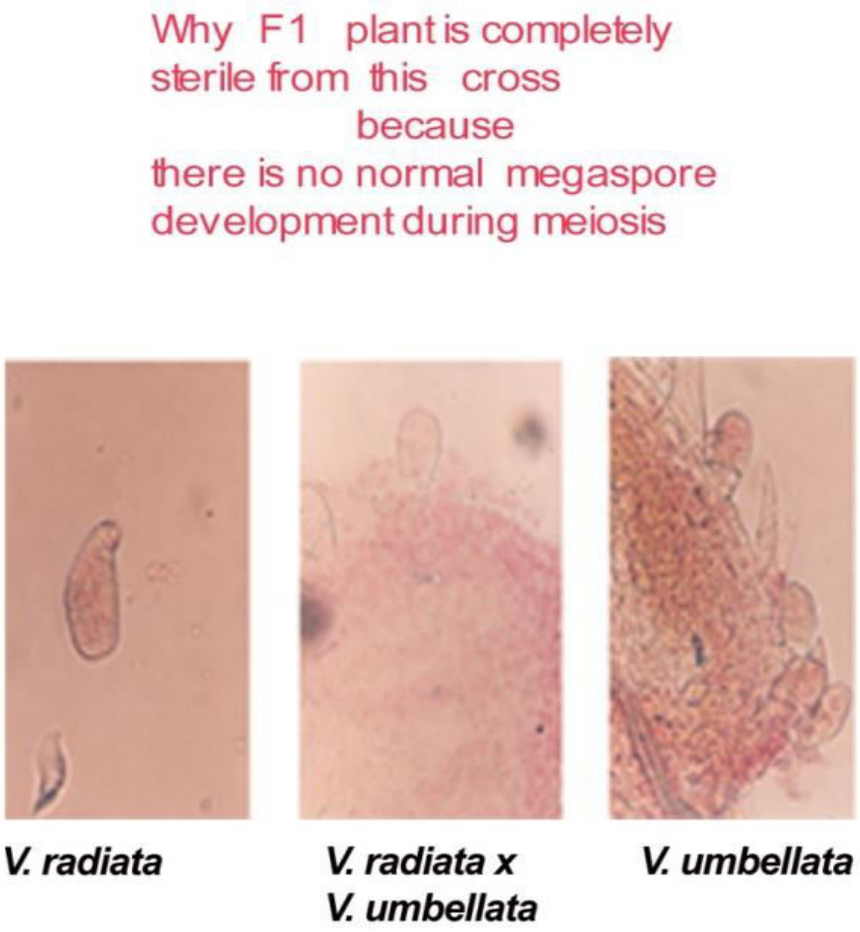


Fig 4. Meiotic abnormalities of embryosac in Vigna radiata x Vigna umbellate and normal megaspore production in parents

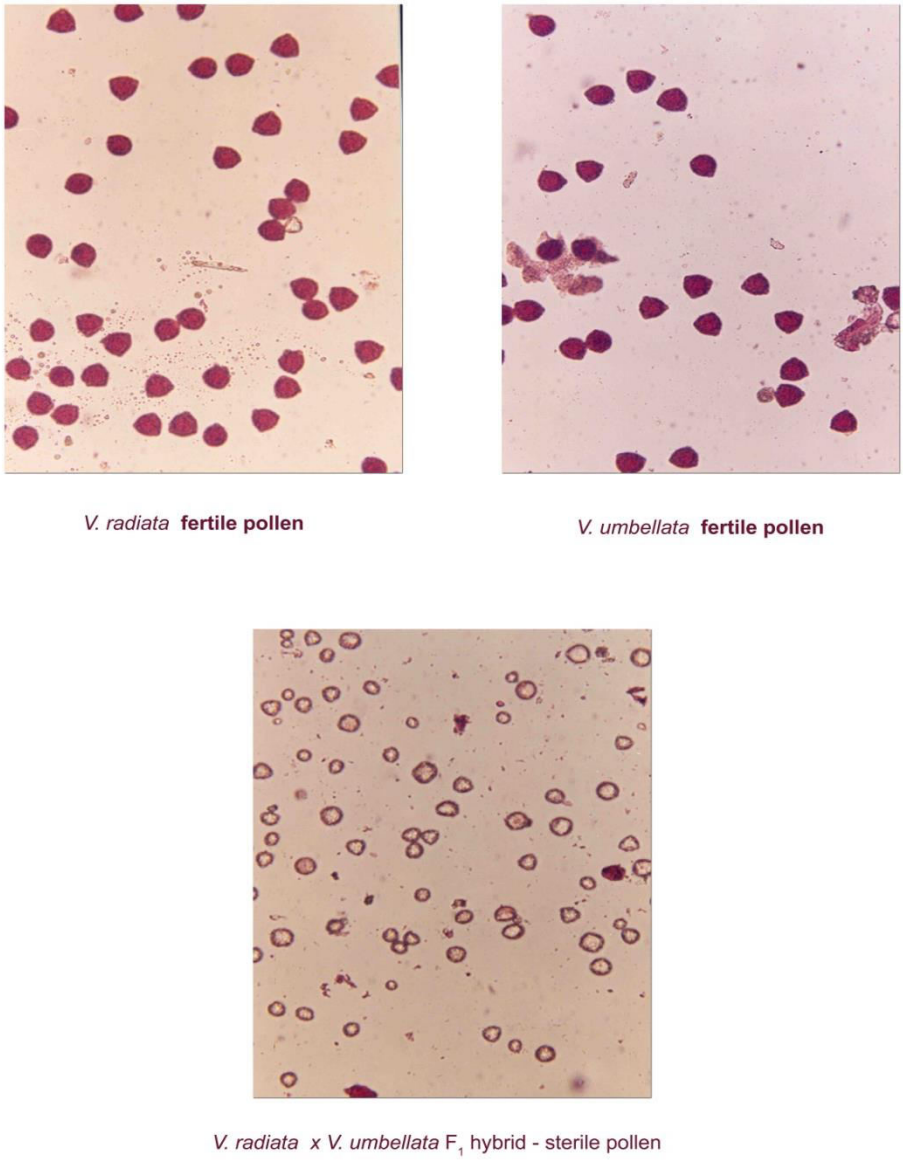
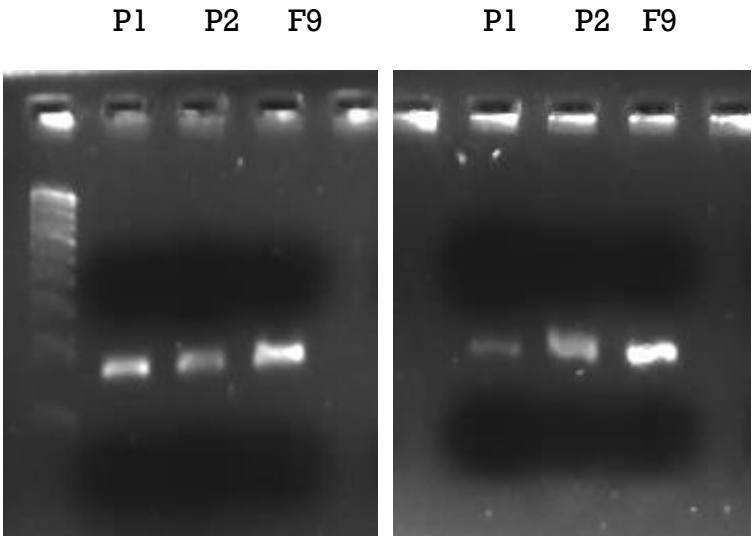


Fig 5. Pollen fertility of Vigna radiata x Vigna umbellata and their parents

PCR analysis for V.radiata x V. umbellata (F9) for finding true hybrid



CEDG 163 GEDG 265

Fig 6. Conformation of true hybrids of Vigna radiata x Vigna umbellata (F9)

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