INDUCTION OF MALE STERILITY IN FINGER MILLET

(*Eleusine coracana* Gaertn.)

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Certificate

This is to certify that the thesis titled **"INDUCTION OF MALE STERILITY IN FINGER MILLET (*Eleusine coracana* Gaertn.)"** submitted by **Miss.Nandini Ramesh**, for the degree of **MASTER OF SCIENCE (AGRICULTURE) in GENETICS AND PLANT BREEDING** of the University of Agricultural Sciences, Bangalore is a record of research work done by her during her study in the University under my guidance and supervision. The thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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INTRODUCTION

I. INTRODUCTION

Finger millet or ragi is an important millet crop of India with an area of 2.2 million hectares producing about 2.6 million tonnes and productivity being 1240 kg per ha is the highest among millets. It is mainly grown for its grain and fodder.

Finger millet growing states in India are Karnataka, Andhra Pradesh, Tamil Nadu, Orissa, Maharashtra, Gujarath, Bihar and Uttar Pradesh. Karnataka ranks first both in production and area. It is grown in an area of 10 lakh hectare with a production of 15 lakh tonnes. It is mostly grown in southern districts of Karnataka.

Systematic work on crop improvement work was initiated as early as 1950. Till 1970's, the genetic improvement in this crop was through pure line selection. In seventies limited hybridization work resulted in a few improved varieties. However, quantum jump in productivity was not achieved mainly because of limited hybridization. Variability available in the germplasm could not be channeled properly due to the difficulties faced in making crosses. The finger millet crop has very small florets and are borne within a spikelet making manual emasculation tedious. Hence most of the hybridization work done so far is through contact method. At present only purple pigmentation is used as marker gene. This imposes a restriction on choice of parents to generate variability in the desired character.

At present there is a plateau in varietal improvement even though a wide range of genetic resources have been accumulated in this crop. High volume crossing utilising the diverse germplasm seems to be the only answer for breaking the yield plateau. As compared to other self and cross pollinated food crop, crop improvement through hybridization is almost negligible.

To alleviate the problem of emasculation faced by the breeders hot water emasculation and use of gametocides were attempted to a limited extent but failed to provide a fool proof methodology which could be utilized successfully in finger millet. Though an indication was got earlier that these methods could be used as potential tools, no sincere attempt was made to standardize the technology so that it could successfully be used. Keeping this in view a series of experiments were conducted to standardize the emasculation technique in finger millet with the following specific objective.

1. To optimise the hot water temperature

2. To fix the duration of exposure that will result in maximum pollen abortion.

3. To identify the most effective treatment concentration of the gametocide.

4. To standardize the stage at which the treatment has to be administered.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Several earlier reports have suggested the use of hot water and growth regulators like Gibberellic acid, Ethrel and 2,4-Dichlorophenoxy acetic acid in inducing male sterility. As a result in the present investigation hot water and growth regulators like GA_3, ethrel and 2,4-D were used to induce male sterility in finger millet. In this chapter a brief review of the past findings of the above have been cited.

2.1. EFFECT OF HOT WATER ON POLLEN

2.1.1. Finger millet

Rama Rao and Rama Rao (1962) successfully emasculated ragi flowers by immersing ears for 10 minutes in hot water at 47°C. Raj *et al.* (1964) obtained best results when the ears were treated with hot water at 52°C for two minutes.

2.1.2. Other Millets

Srivastava and Yadav (1972) reported successful emasculation of little millet (*Panicum miliare*) florets by immersing ears at 49°C for 8-10 minutes or at 50°C for 5 minutes without any damage to female organ.

2.1.3. Cereals

Satake and Yoshida (1978) reported that high temperature of more than 32°C at flowering reduced seed set in indica rice.

Bardhan Roy and Biswas (1982) reported an average ambient temperature of 25°C from panicle initiation to flowering adversely affected spikelet fertility in rice varieties.

Imaki *et al.* (1983) reported 0-2 per cent grain set in rice in glass house when the temperature was 39°C for 6 hours. They noticed that anther dehiscence was inhibited by high temperatures.

Naidu and Reddy (1988) observed spikelet sterility in the range of 4.3 per cent in rice (IR 20) to 28.88 per cent (IR 50) due to high temperatures during panicle initiation to flowering.

Tan *et al.* (1988) noticed that spikelet sterility in two hybrids of rice Shan You 2 and Wei You 2 increased as temperatures increased beyond 35°C and as the time of exposure increased from 2-6 hrs.

2.2. EFFECT OF GIBBERELLIC ACID

2.2.1. Effect of GA_3 on pollen sterility and seed fertility

2.2.1.1. *Finger millet*: Suryanarayan Reddy *et al.* (1983) reported that application of 250 ppm of GA_3 to the inflorescence of ragi variety Hamsa gave maximum pollen sterility (93.05%) followed by 300 ppm (75.85%), 150 ppm (75.46%) and 100 ppm (29.54%) as against 5.33 per cent in control. The treatments were given twice daily at 9 a.m. and 3 p.m. for three days.

2.2.1.2. *Millets*: Shivaramaiah (1985) reported 80 per cent pollen sterility when the plants of common millet were sprayed thrice with GA_3 on 35th, 40th and 45th day after sowing.

2.2.1.3. *Cereals*: Nelson and Rossman (1958) reported spraying of 500 to 1000 ppm GA_3 to maize resulted in partial sterility. This was confirmed under field trial using early flowering inbred line R 53 and late flowering inbred line OH 51. Early and late inbred lines were sprayed with 100, 1000 2000 and 2500 ppm of potassium Gibberellate. Early inbred received a dosage at 1 to 10 mg per plant while late inbred received 12 to 35 mg per plant. Gibberellin induced male sterility was 100 per cent.

Zdril'Ko (1963) reported 100 per cent male sterility in several maize varieties and hybrids when treated with 0.5 per cent solution of Gibberellin.

Bose and Sharma (1972) reported that pollen sterility in rice increased with the increase in concentration of GA_3 in a combined treatment of Gibberellic acid and Colchicine.

Hansen et al (1976) obtained complete barren tassels when two inbred lines of maize A 619 and *Gaspe* were treated with GA_3 three days prior to the start of meiosis. Changes in floral components and internode elongation were also noticed.

Hughes *et al.* (1978) reported successful use of GA_3 in combination with ethephon for induction of male sterility in winter Cv. *Nimsod* of wheat The treatment with GA_3 at the rate of 12.8 kg a.i. per ha in the combination with 6.4 kg ethephon a.i. per ha given before meiosis induced maximum male sterility.

Radley (1980) found that Gibberellic acid treatment prevented grain setting in all florets of tall and semidwarf wheat cultivars.

2.2.1.4. *Other crops*: Schuster (1969) treated sunflower plants at the beginning of bud development with 0.25 to 0.50 mg per plant of GA_3 and observed 90 to 100 per cent male sterility. The inbred line "HEBH" was also treated with GA_3 and the seed set ranged from 0 to 86 per cent as compared to 90 per cent in the untreated plants. Different genotypes responded differently to the treatment under different climatic conditions.

Anascenko (1972) reported that 0.005% GA_3 solution to cause male sterility in sunflower. Changes in morphological characters like decrease in the diameter of the capitulum and number of flowers were also observed.

Klimov (1973) reported that 0.005% aqueous solution of Gibberellic acid causes male sterility in sunflower variety Peredovik.

Seetharam and Kusumakumari (1974) reported that application of GA_3 on the 3rd day after flower bud initiation induced maximum pollen sterility in sunflower and the extent of pollen sterility decreased as the time of treatment was delayed. Thus, the stage of application is an important critical factor. They also reported that 90 to 100 ppm of GA_3 was sufficient to induce 100 per cent male sterility and for induction of male sterility on a mass scale, spraying of plants with 100 ppm GA_3 at the rate of 0.5 to 0.75 ml per plant on the 30th and 40th day after sowing was found suitable.

Spirova (1975) successfully induced male sterility in four inbred lines of sunflower when 0.5 ml GA was applied at 5 to 6 leaf stage and at the flower bud stage.

Seetharam and Kusumakumari (1976) reported degeneration of sporocytes before entering meiosis in GA_3 treated plants of sunflower. Abnormalities and disorganization in the tapetal cells caused starvation of meiocytes which in turn resulted in abortion.

Bose and Nitsch (1970) reported inhibition of male flowers and induction of female flowers on the first 10 nodes of GA_3 treated plants of huffa acutangula.

Davidyan (1973) found 100 per cent monoecious plants in the northern varieties of hemp plants treated with 0.01 to 0.2 per cent Gibberellin before bud initiation.

Vander Meer and Van Bennekom (1973) reported that dry bulbs of onion treated with 1% solution of Gibberellic acid to induce male sterility. Plants treated with 0.5 to 2% GA_3 at the rate of 2.5 ml per plant on 47th day recorded partial male sterility.

Pavithran and Ravindranathan (1976) found that foliar application of GA_3 to cashew trees increased the number of flowers per inflorescence and reduced the ratio of hermophrodite to male flowers.

2.2.2. Effect of GA_3 on morphological characters

2.2.2.1. *Millets*: Malali *et al.* (1981) reported the application of Gibberellic acid to female sorghum parent (2077A) hastened flowering by 5.53 days. Vadivelu *et al.* (1984) found that treating sorghum male sterile 2077 with GA at 220 ppm advanced flowering by 3-7 days while treatment with C.C.C. (chloromequat) at 300 ppm or maleic hydrazide at 500 ppm delayed the flowering of restorer CS 3541 by 5 days over the untreated controls.

Rood *et al.* (1988) reported that the exogenous application of GA_3 promoted height, growth and inhibited tillering particularly when the application was made during rapid vegetative growth of sorghum. Applications of GA_3 subsequent to panicle initiation promoted panicle elongation but did not alter the time of anthesis.

2.2.2.2. *Cereals*: Singh *et al.* (1980) reported that dwarf and semidwarf mutants of wheat did not show increase in culm length at maturity in response to exogenous applications of 150 and 250 µg/ml GA_3.

Pereira *et al.* (1991) reported 100 ppm GA increased plant height in wheat Cv. BR 501 and reduced plant height in BR 503.

Rajni *et al.* (1992) reported that vigour index of cultivar Basumathi 370 rice was adversely affected whereas semidwarf and dwarf cultivars responded positively when treated with 10, 25 and 50 µg ml^{-1} of GA_3. Root length decreased in Cv. Basumathi 370 when subjected to high concentration (50 µg ml^{-1}) of GA_3 but increased in other cultivars. Maximum increase in root and shoot length (%) was recorded invariably in all the cultivars following GA_3 treatment.

Thangaraj and Sivasubramaniam (1992) found that the total dry matter and panicle number increased in all the varieties of rice when treated with foliar spray of Kinetin 20 ppm at heading followed by GA_3 25 ppm at panicle initiation and tender coconut water 2% at panicle initiation and heading.

2.2.2.3. *Other crops*: Podesva and Sebanek (1986) reported promotion of seedling growth of pea *Lepidium sativum* when treated with 500 mg GA_3 per litre.

2.3. EFFECT OF ETHREL

2.3.1. Effect of ethrel on pollen sterility and seed fertility

2.3.1.1. *Millets*: Savchuck (1979) observed 83.6 per cent male sterility in Panicum miliaceum Cv. Mironovk 94 treated with 2 per cent ethrel

2.3.1.2. *Cereals*: Rowell and Miller (1971) reported absence of seed set in wheat plants treated with 1000 to 3000 ppm of ethrel at pre-boot and boot leaf stages. With advancing maturity the concentration of ethrel needed to approach 100 per cent sterility also increased.

Bennet and Hughes (1972) observed high pollen sterility in *Triticum aestivum* var. Sirius when treated with ethrel at a concentration of 2000 ppm and above. In male sterile florets, anther development was abnormal, size was reduced and extrusion and dehiscence of anthers failed.

Sen (1972) recorded male sterile plants in ethrel treated plants of wheat and barley. The concentrations of ethrel used was 0, 500, 1000 and 2000 ppm at pre-booting and post booting stage. They also observed reduction in plant height and 100 grain weight. The most effective concentration was 100-200 ppm.

St Pierre and Trend (1972) reported that two applications of ethrel induced male sterility in wheat.

Borghi *et al.* (1974) observed complete sterility when *Triticum aestivum* varieties Flamingo and S.Pastore and *Triticum durum* variety lambo were treated with 2000 ppm of 2-Chloro Ethane Phosphoric acid spray at early, mid, late booting and early heading stages.

Hughes *et al.* (1974) reported that treatment of ethrel before meiosis to Sirius variety of wheat induced maximum male sterility. Foliar application of GA_3 at 100, 200 or 300 ppm 2-5 days after treating the plants with 2000 ppm ethephon before the end of pre-meiotic interphase of archisporial development resulted in full male-sterility. When ear emergence was complete, application of 6.4 to 12.8 kg per ha a.i. of ethephon followed by 1.1 kg per ha a.i. of GA_3 three days later induced male sterile ears.

Doltacil and Apltanerova (1977) reported that repeated applications of ethrel at 0.09% during the 9th developmental stage in spring wheat was effective in causing a maximum of 49 per cent sterile pollen grains. Very little seed set of 0.16 seeds per spikelet was observed in another experiment when the plants were treated with 0.24 per cent ethrel solution at 9th or 10th developmental stage.

Doltacil and Apltanerova (1980) found that 39.5 per cent ethephon was more effective as a male gametocide than 50 per cent camposan in wheat.

Leonova (1980) reported that wheat plants treated with 0.1 per cent etheptron exhibited 3.5 to 6 per cent grain set and 50 to 70 per cent retardation of growth, while 10 per cent growth retardation and 0.4 to 7 per cent grain set was observed in 0.3 per cent ethephon treated plants.

Wang and Que (1981) reported that treatment of rice plants with 300 ppm of Ethrel resulted in 86.5 per cent male sterility and partial male sterility when treated with 100 ppm of ethrel and complete sterility, when the dose was increased to 500 ppm.

Rathgeb *et al.* (1982) reported high percentage of male sterility in secondary ears of hexaploid triticale when treated at three development stages with ethrel or DPX 3778. They also observed 87.5 per cent male sterility in primary ears and 84.5 per cent male sterility in secondary ears treated with 4000 ppm ethrel. Increase in plant height by 22 per cent was also recorded.

Law and Stoskopf (1973) reported 96 per cent male sterility in barley plants treated with ethrel at the time of panicle primordial development stage. Under field conditions male sterility upto 55 per cent was induced when 1.4 kg per ha of ethrel was applied at the flag leaf emergence stage.

Perez *et al.* (1973) found that spraying of ethrel at the early booting stage induced high pollen and spikelet sterility and reduced the number of spikelets per panicle and the length of the flag leaf in rice.

Nelson (1975) reported that out of three varieties of triticale treated with ethrel from 0 to 3000 ppm, 3000 ppm was very effective in one variety when it was applied at boot leaf stage in causing male sterility. However under field situations on different varieties of triticale application of 0 to 2000 ppm ethrel was ineffective in causing male sterility.

2.3.1.3. *Other crops*: Borghi and Pironi (1970) reported that two applications of ethrel at 1 or 2 and 3 to 4 true leaf stages at concentrations of 600 to 800 ppm in *Cucurbita pepo* and 500 to 600 ppm in cucumber caused male sterility. Ethrel reduced the number of male to female flowers in both.

Tarakanov and Ayapova (1973) reported that varieties of cucumber when treated with ethrel at two leaf stage, depressed growth and caused a shift towards female sex.

Sulikeri and Bhandary (1973) found that application of 500 ppm ethrel at first true leaf stage caused maximum delay in the appearance of staminate flowers of melons and squashes. The ratio of male to female flowers was 22.3:1 in control. 250 ppm of ethrel treatment produced the highest number of female flowers.

Singh and Choudhury (1988) reported that the induction of pistillate flowers earlier and at lower nodes in cucumber (*Cucumis sativus*) and bottle gourd (*Legenaria siceraria*) delayed the appearance of female flowers in watermelon (*Citrulus lunatus*) when they were treated with 50 and 1000 ppm of ethrel. Ethrel at 50 ppm produced the lowest sex ratio in all the three crops.

Vadigeri and Malageri (1989) reported a significant increase in the number of female flowers of poinselte and Belgaum local seedlings of cucumber when treated with 400 ppm of ethrel.

2.3.2. Effect of ethrel on morphological characters

2.3.2.1. *Millets*: Pereira *et al.* (1991) reported that 100 ppm ethephon reduced plant height in all cultivars of sweet sorghum. But 100 ppm GA increased plant height in cv. BR 501 and reduced plant height in BR 503.

2.3.2.2. *Cereals*: Leonova (1980) reported that wheat plants treated with 0.1 per cent ethephon exhibited growth retardation of 50 to 70 per cent while growth retardation upto 10 per cent was recorded when treated with 0.3 per cent ethephon.

2.3.2.3. *Other crops*: Tarakanov and Ayapova (1973) reported depressed growth of cucumber when treated with ethrel at two leaf stage.

Jayaram and Ramaiah (1980) reported significant reduction in percentage of flower shedding in cowpea when treated with a combination of 25 kg P_2O_5 per ha, Planofix at 40 ppm and ethrel at 100 and 150 ppm.

2.4. EFFECT OF 2,4-D

2.4.1. Effect of 2,4-D on pollen sterility and seed fertility

2.4.1.1. *Cereals*: Aswathanrayana and Mahadevappa (1992) reported that in rice all the treatments viz., 200-3000 ppm GA_3, 500-8000 ppm Ethrel (ethephon), 0.1 to 1.6 per cent 2,4-D or 0.05 to 0.8 per cent maleic hydrazide induced pollen sterility and decreased seed set compared to untreated control. Highest pollen sterility was induced with 800 ppm GA_3, 8000 ppm ethrel (68.60%), 0.8 per cent 2,4-D (61.95%) and 0.2 per cent MH (86.00%).

2.4.2. Effect of 2,4-D on morphological traits

2.4.2.1. *Cereals*: Brasesco (1976) found ear abnormality in Precoz Parana INTA wheat due to 2,4-D treatment.

Lyaskovskii (1990) reported 18-26 per cent reduced plant height in all cultivars of wheat and barley and 10-21 per cent increased diameter of the 2nd internode when plants were treated with slow release fertilizers that contained 2,4-D as one of the constituents.

Yaduraj and Ahuja (1992) reported that 8 varieties of wheat when treated with 2,4-D (0.6 and 1.2 a.i. kg/ha) and applied at three different dates (4, 5 and 6 weeks after sowing) developed ear deformity and the most severe deformity was recorded with 2,4-D application of 1.2 kg per ha 5 weeks after sowing 2,4-D applied 4 weeks after sowing significantly reduced plant height.

2.4.2.2. *Other crops*: Chauhan and Bordia (1971) reported that there was no effect on plant height or in number of days taken for seed fertility in plants raised from soaked seeds of cabbage for 24 h in solutions of 5-100 ppm GA_3

5-150 ppm NOA or 0.5-5 ppm 2,4-D. 2,4-D treatment increased the number of edible leaves and head weight increased to a maximum of 1.72 kg with 50 ppm GA.

Podesva and Sebanek (1986) reported inhibition of seedling growth of pea, *Lepidium sativum* and barley when treated with 200 mg 2,4-D per litre while treatment with 500 mg GA per litre promoted seedling growth.

Patil *et al.* (1992) reported 0, 1, 5, 25 or 500 ppm 2,4-D sprayed 30 DAS to cotton cv. Sharada decreased stem and leaf dry weight at 52 and 72 DAS. Spraying GA_3 increased plant height.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The investigations were carried out at the Main Research Station, Gandhi Krishi Vignana Kendra, University of Agricultural Sciences, Bangalore which is located at latitude and longitude of 12°58'N and 77°35'E, respectively, and has an altitude of 930 metres above mean sea level.

The materials and methods required to conduct the following four experiments:

1. Immersion of finger millet panicles in different temperature regimes of hot water ranging from 45-50°C for a standard duration of 5 minutes.

2. Immersion of finger millet panicles in 48-49°C hot water for different durations ranging from three minutes to nine minutes.

3. Application of different concentrations of GA_3, ethrel and 2,4-D.

4. Application of 1600 ppm GA_3 and 100 ppm 2,4-D at five different growth stages of finger millet.

3.1. MATERIALS AND METHOD

For all the four experiments finger millet Cv. PR.202, was selected. PR.202 is a medium tall variety with compact ears and matures in about 115-120 days. The nursery was raised and plants were transplanted into pots.

3.1.1. Experiment I: Hot water emasculation by immersing finger millet panicles in different temperature regimes of hot water ranging from 45-50°C for 5 minutes

Seedlings of Cv. PR.202, were raised during the summer season of 1995-96 and transplanted into pots. The procedure adopted for this study was that three tillers of Cv.PR.202 of the same age which are likely to flower during the next 2-3 days were selected. In each ears all but three fingers were removed and immersed into a beaker containing 45-46°C hot water for 5 minutes. Similar procedure was carried out for the other hot water temperature regimes of 46-47°C, 47-48°C, 48-49°C and 49-50°C. The following observations were recorded from all the three treated fingers of each treatment.

1. *Pollen sterility*: Three spikelets each from the tip, middle and basal portion of each treated finger was collected separately. Further, from each of the three spikelets the mature anthers of the apical and basal florets were smeared in 2% acetocarmine and assessed for pollen fertility. Three fields per slide were scored. Round darkly stained and plumpy pollen grains were counted as fertile and lightly stained and shrunken were counted as sterile pollen grains. Pollen fertility/sterility was expressed in percentage.

2. *Seed fertility*: This observation was recorded at complete maturity. In each of the treated tillers the seed fertility was determined by collecting spikelets from the tip, middle and basal portions of the finger. Number of seed set in each spikelet was counted. Seed fertility was expressed in percentage.

3.1.2. Experiment II: Immersion of finger millet panicles in 48-49°C hot water for different durations ranging from 3.0 minutes to 9.0 minutes

Based on the results of Experiment I immersion of fingers in hot water of 48-49°C for different durations of time namely 3.0, 4.5, 6.0, 7.5 and 9.0 minutes were adopted. Hot water treatment procedure adopted in Experiment I was slightly modified. Instead of beaker, a thermoflask of one litre capacity with a lid made of thermocool was used.

The observations recorded in Experiment I were repeated in this experiment also.

3.1.3. Experiment III: Chemical emasculation using different concentrations of GA_3, ethrel and 2,4-D

Seedlings of PR.202 raised during kharif season of 1995-96 were transplanted into pots. Pot experiments were conducted. Aqueous solutions of different concentrations of GA_3, ethrel and 2,4-D were prepared as in table below. Totally 75 tillers were ear marked for this study. For each chemical 25 tillers were used. Each concentration of each chemical was applied on five tillers to serve as five replications. Various concentrations of gametocides were applied when the junctures of top 2-3 leaves including flag leaf were invisible.

For each of the tiller a capillary tube was inserted in the leaf whorl. With the help of a pipette 1 ml of the required concentration of the gametocide was pipetted out and poured into the capillary tube. Each of the concentration of the chemical was applied to 5 tillers. The following are the different concentrations of the three gametocides used and the number of tillers treated in the study.

Treatment	Concentration of GA3 in parts per million	Concentration of ethrel in parts per million	Concentration of 2,4-D in parts per million	No of tillers treated
T_1	100	1000	25	5
T_2	200	2000	50	5
T_3	400	3000	100	5
T_4	800	4000	200	5
T_5	1600	5000	400	5

Following were the observations recorded.

1. <u>Pollen sterility</u>: As mentioned in section 3.1.2.1.

2. <u>Seed fertility</u>: As mentioned in section 3.1.2.1.

3. Plant height: This observation was recorded from the base of the plant to the tip of the panicle and expressed in centimeters.

4. Flag leaf length: Length of flag leaf from the tip of the leaf blade to the base of leaf blade was recorded in centimeter.

5. Flag leaf breadth: The breadth at the middle of the flag leaf blade was recorded in centimeters.

6. Finger length: Is measured in centimeters from the tip of the finger to the base of the finger at complete maturity.

3.1.4. Experiment IV: Application of 1600 ppm GA_3 and 100 ppm 2,4-D at five different leaf stages of finger millet

Based on the results of Experiment III 1600 ppm GA_3 and 100 ppm 2,4-D were selected on these concentrations induced highest pollen sterility and least seed set. Seedlings of PR.202 raised during the rabi season of 1995-96 were transplanted into pots. Totally 50 tillers were used for each chemical. One concentration of each chemical was applied at five growth stages. First leaf stage refers to the growth stage in which the junction of flag leaf alone is invisible while 2nd, 3rd, 4th and 5th leaf stage refers to the growth stage in which junctures of 2nd, 3rd, 4th and 5th leaf juncture below flag leaf including flag leaf are invisible.

Observations recorded were in the same manner as in Experiment III.

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

The results of the current investigations are presented under the following heads.

1. Experiment I: Hot water emasculation using different temperature regimes.

2. Experiment II: Hot water emasculation using 48-49°C hot water for different intervals of time ranging from 3 to 9 minutes.

3. Experiment III: Effect of different concentrations of GA_3, ethrel and 2,4-D.

4. Experiment IV: Effect of 1600 ppm GA_3 and 100 ppm 2,4-D applied at 5 different leaf stages of finger millet.

4.1. EXPERIMENT I: HOT WATER EMASCULATION USING DIFFERENT TEMPERATURE REGIMES

4.1.1. Pollen sterility and seed fertility

The data on pollen sterility and seed fertility are presented in Table 1. From the table it is seen that all the treatments recorded higher pollen sterility than the control. With the increase in temperature from 45° to 49°C pollen sterility increased from 42.1 per cent to 67.9 per cent, respectively

Table 1. Effect of treatment of finger millet ears before anthesis with hot water of different temperatures on pollen sterility and seed fertility

Temperature range in degrees centigrade	Mean pollen sterility						Mean pollen sterility	Mean seed fertility			Mean
	Spikelets at tip of the finger		Spikelets at middle of the finger		Spikelets at base of the finger			Spikelets at tip of the finger	Spikelets at middle of the finger	Spikelets at base of the finger	
	Apical floret	Basal floret	Apical floret	Basal floret	Apical floret	Basal floret					
45-46	50.6	51.1	45.5	45.8	48.8	36.7	42.1	100.0	100.0	100.0	100.0
46-47	43.9	42.5	59.3	58.2	54.5	53.4	51.9	50.0	90.9	100.0	80.3
47-48	66.0	60.4	60.8	58.4	65.7	60.5	61.9	-	-	-	-
48-49	72.9	70.9	65.5	63.6	66.5	68.2	67.9	0.0	20.0	0.0	6.6
49-50	Dried up							Dried up			
Control (without treatment)	6.3	5.2	5.3	5.2	6.0	5.6	5.6	100.0	100.0	100.0	100.0
Mean over apical and basal florets	57.3		57.1		56.75			50.0	70.3	66.6	

Mean of apical florets of tip, middle and base of finger = 58.3
Mean of basal florets of tip, middle and base of finger = 55.8

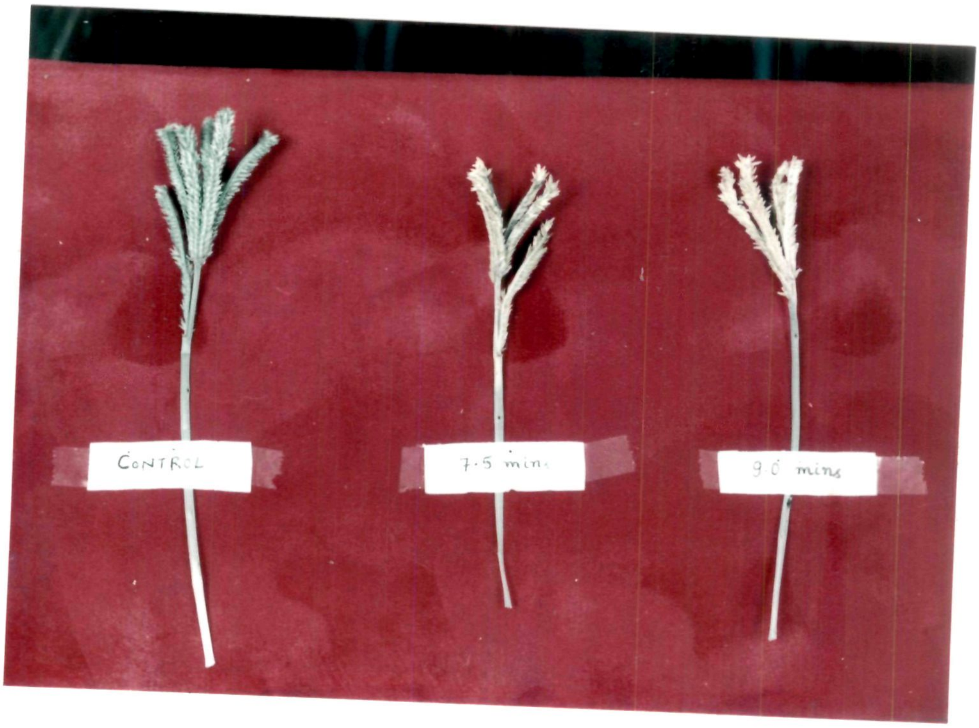


Plate 1 Effect of 48-49°C hot water for immersion durations of 7.5 and 9.0 minutes in drying up of panicles

The same is depicted in Figure 1. Increase in water temperature beyond 49°C had a deleterious effect resulting in drying off the ears (Plate 1). There was not much of a difference in pollen sterility between the tip, middle and basal positions of fingers, whereas sterility differences between apical and basal florets within a spikelet were observed.

Seed fertility showed an increasing trend with the decreasing temperatures ie., with the increase in temperature ranges seed fertility reduced. However, temperature ranges from 45-47°C did not affect seed fertility, while seed fertility at 47-48°C could not be recorded. But there was a drastic reduction in seed set from 100 per cent to 6.6 per cent at 48-49°C. Further increase in water temperatures resulted in drying of the ear.

4.2. EXPERIMENT II: HOT WATER EMASCULATION USING 48-49°c HOT WATER FOR DIFFERENT INTERVALS OF TIME RANGING FROM 3 TO 9 MINUTES

4.2.1. Pollen sterility and seed fertility

The results are presented in Table 2. As per the table, the duration of immersion of ragi ears increased from 3.0 to 6.0 minutes pollen sterility also increased. The same is shown in Figure 2. However, further increase in immersion beyond 6.0 minutes had deleterious effect on the panicle resulting in total burning. The shortest duration of three minutes immersion caused significant amount of sterility (66.5%) as against control (5.4%). A maximum pollen sterility of 82.7 per cent was recorded when the ears were immersed

Table 2. Effect of 48-49°C hot water on pollen sterility and seed fertility of finger millet ears

Duration of treatment in minutes	Mean pollen sterility: Spikelets at tip of the finger – Apical floret	Spikelets at tip of the finger – Basal floret	Spikelets at middle of the finger – Apical floret	Spikelets at middle of the finger – Basal floret	Spikelets at base of the finger – Apical floret	Spikelets at base of the finger – Basal floret	Range	Mean pollen sterility	Mean seed fertility: Spikelets at tip of the finger	Spikelets at middle of the finger	Spikelets at base of the finger	Range	Mean
3.0	61.1	68.4	71.9	45.7	76.5	75.4	31.7-100.0	66.5	36.4	[illegible]	18.2	18.2-30.0	24.8
4.5	75.7	75.0	77.0	73.5	87.5	77.0	63.3-100.0	77.6	9.1	[illegible]	8.3	10.0-27.2	9.1
6.0	93.5	76.7	85.9	72.1	90.2	78.2	63.6-100.0	82.7	0.0	[illegible]	8.3	0.0-16.6	6.1
7.5	Dried up								Dried up				
9.0	Dried up								Dried up				
Control (without treatment)	5.9	5.5	5.4	5.0	5.8	5.0		5.4	95.9	[illegible]	100.0		98.3
Mean over apical and basal florets	75.1		71.1		80.8				15.2	13.3	11.6		

Mean of apical florets of tip, middle and base of finger = 79.9
Mean of basal florets of tip, middle and base of finger = 71.3

Fig.1. Effect of different temperatures of hot water on pollen sterility and seed fertility

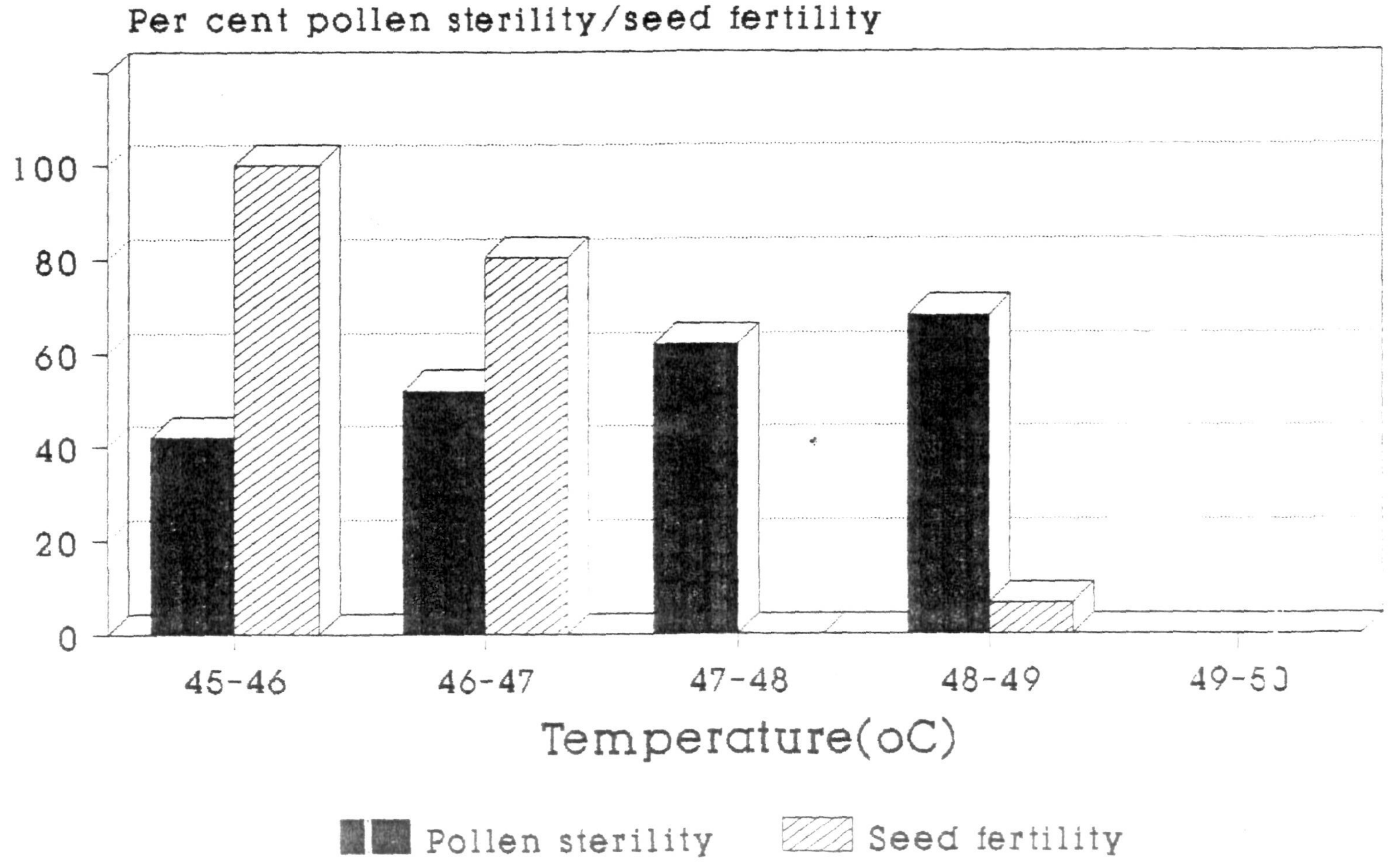
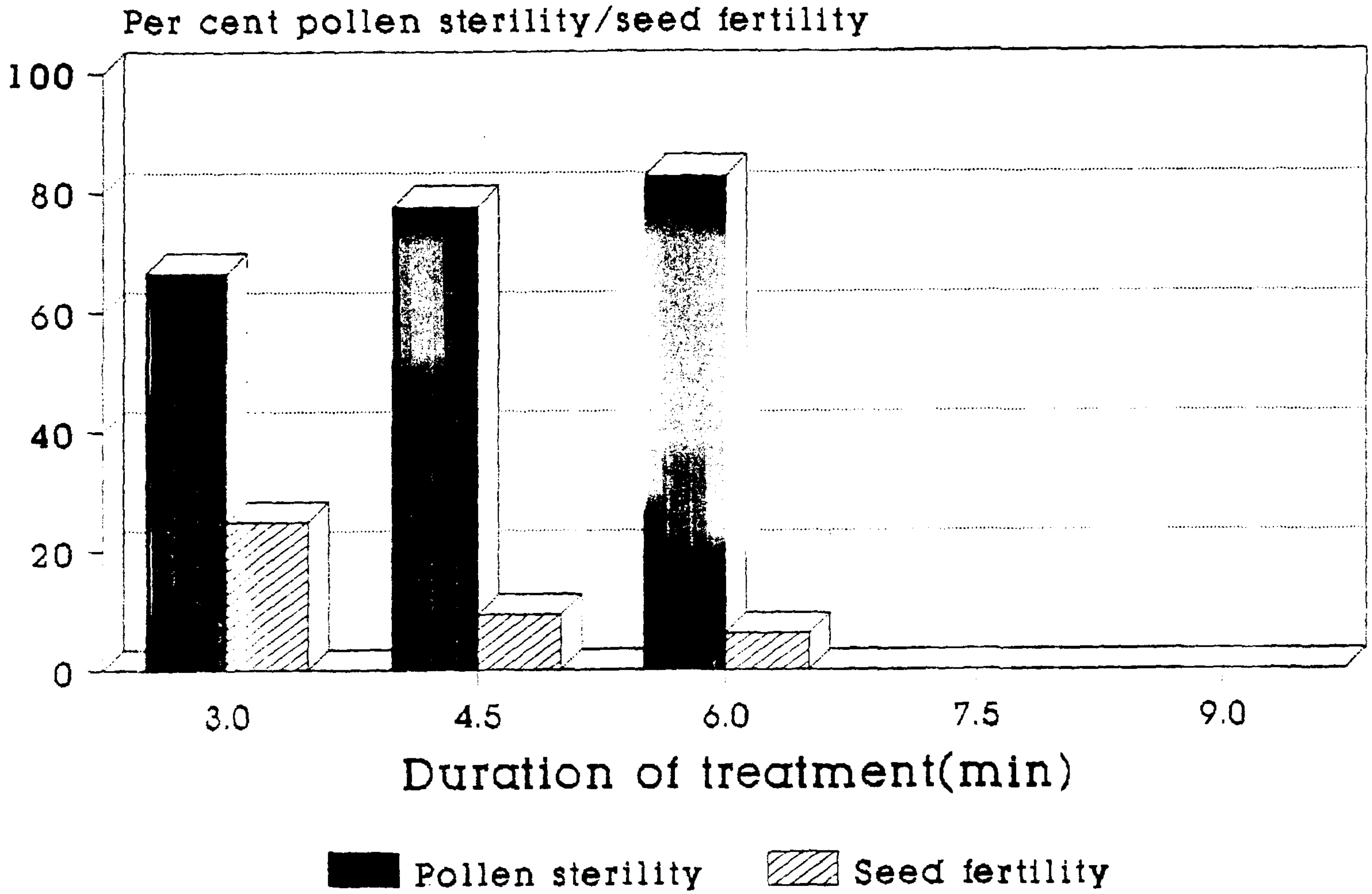


Fig.2. Effect of 48-49oC hot water on pollen sterility and seed fertility



for 6.0 minutes. Basal parts of finger was affected to the maximum extent than the middle. Apical florets within a spikelet was more affected than basal florets.

Seed fertility increased with reduced immersion time. The seed fertility got reduced from 98.3 per cent (control) to 20.0 per cent when the ear was immersed for 3.0 minutes. The least fertility of 6.1 per cent was recorded at the immersion duration of 6.0 minutes. Least seed set was recorded in the basal fingers than apex or middle portion.

Similarly mean pollen sterility range was narrowed down with the increase in duration of immersion. However, the maximum pollen sterility of 100 per cent was recorded in all the duration of immersion. In contrast the seed fertility range increased with the increase in immersion time. Zero fertility was only recorded at an immersion time of six minutes.

4.3. EXPERIMENT III: EFFECT OF DIFFERENT CONCENTRATIONS OF GA_3, ETHREL AND 2,4-D

4.3.1. Effect of different concentrations of GA_3

4.3.1.1. Pollen sterility and seed fertility

Results of the effect of different concentrations of GA_3 on pollen sterility and seed fertility are tabulated in Table 3. The results indicate an increase in sterility with increase in concentration of GA_3 from 100-1600

Table 3. Effect of different concentrations of GA_3 on pollen sterility and seed fertility in finger millet treated at 2-3 leaf stage

Concentrations of GA_3	Mean pollen sterility						Mean pollen sterility	Mean seed fertility			Mean
	Spikelets at tip of the finger		Spikelets at middle of the finger		Spikelets at base of the finger			Spikelets at tip of the finger	Spikelets at middle of the finger	Spikelets at base of the finger	
	Apical floret	Basal floret	Apical floret	Basal floret	Apical floret	Basal floret					
100 ppm	25.0	15.2	22.0	17.1	17.5	14.3	18.5	94.4	100.0	85.0	93.2
200 ppm	29.5	19.5	26.1	21.3	25.5	21.8	23.9	68.7	62.5	72.7	67.9
400 ppm	36.4	30.2	32.6	28.6	33.7	31.1	27.3	61.1	63.2	70.0	64.7
800 ppm	43.5	40.9	34.9	33.3	43.6	40.4	32.7	53.3	60.1	58.3	57.2
1600 ppm	51.2	43.2	44.4	40.5	52.1	50.0	46.9	42.1	50.0	44.4	45.5
Control (No treatment)	6.0	5.8	6.2	5.4	5.5	5.9	5.8	98.6	99.0	95.0	97.5
Mean over apical and basal florets	33.4		30.1		33.0			63.9	67.2	66.1	

Mean of apical florets of tip, middle and base of finger = 34.5
Mean of basal florets of tip, middle and base of finger = 29.8

ppm. This can be seen from Figure 3. Not much effect on sterility was noticed at 100 ppm while 200 and 400 ppm caused sterility to a considerable extent. At 800 ppm and 1600 ppm maximum sterility was observed. Apical and basal positions of fingers exhibited more sterility than the middle portion. Within the spikelets apical florets were more prone to sterility than basal florets.

Seed fertility was not affected at 100 ppm of GA_3 while concentrations of 200 and 400 ppm affected seed set. Further reduction of seed set was observed at 800 ppm, at 1600 ppm the seed set was least (45.5%). Seed set recorded in middle and basal spikelets of the fingers were more than the apical spikelets.

4.3.1.2. Morphological traits

Results on the effect of different concentrations of GA_3 on morphological traits are presented in Table 4.

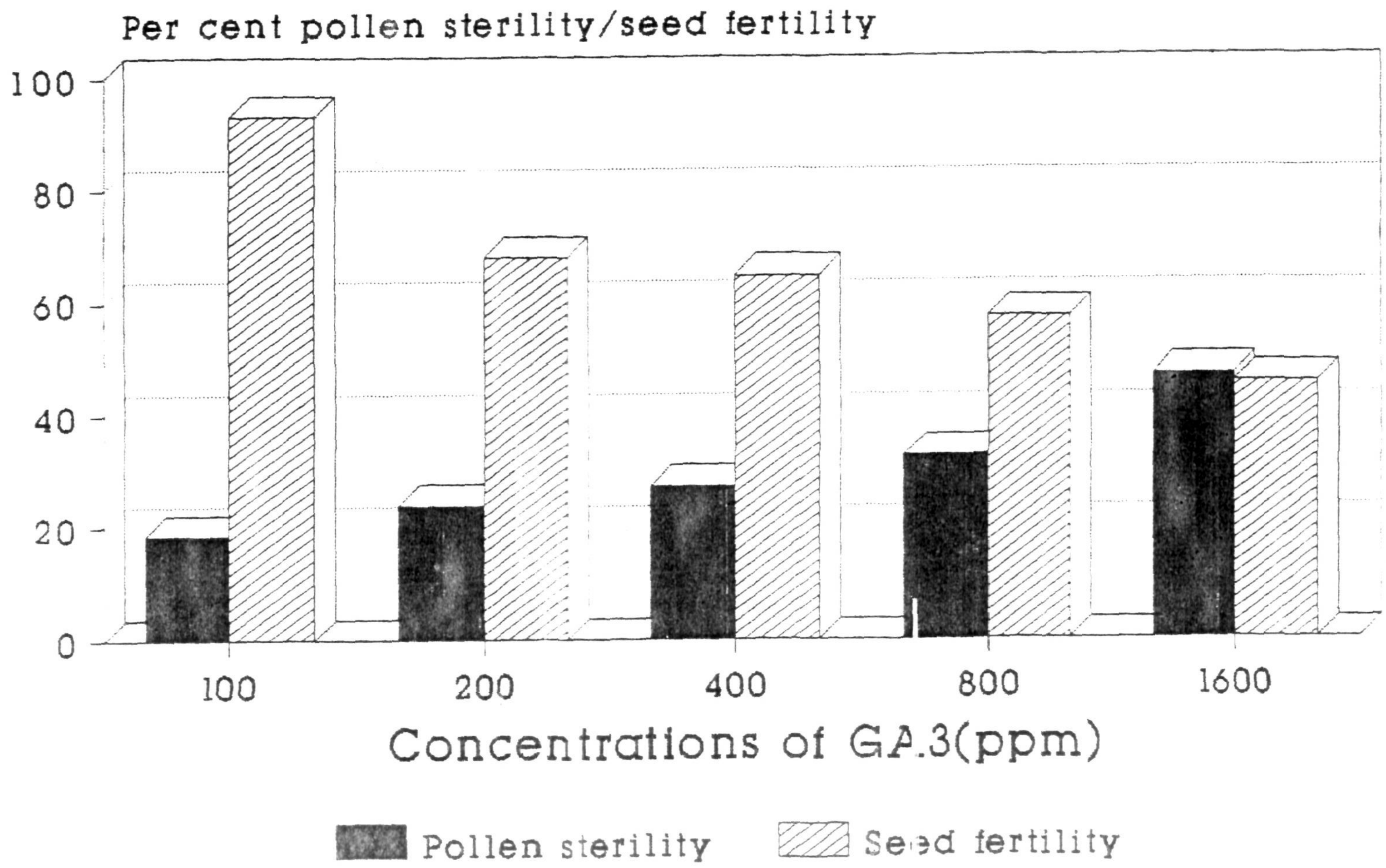
4.3.1.2.1. Plant height

Lower concentrations of 100 and 200 ppm of GA_3 did not affect the height. However with the further increase in concentration of GA_3 affected the height significantly. The highest concentration of 1600 ppm of GA_3 increased the plant height from 112.8 cm (control) to 146.6 cm.

Table 4. Effect of different concentrations of Gibberellic acid on morphologica characters of finger millet

Concentration	Mean plant height (cm)	Mean peduncle length (cm)	Mean Flag leaf		Mean finger length (cm)
			Length (cm)	Breadth (cm)	
100 ppm	113.4	21.2	26.3	1.3	5.9
200 ppm	119.4	21.1	25.5	1.3	7.3
400 ppm	121.4	21.2	31.1	1.3	7.1
800 ppm	133.8	22.5	32.1	1.2	9.1
1600 ppm	146.6	24.3	34.2	1.1	10.1
Control (No treatment)	112.8	20.5	29.6	1.4	5.7

Fig.3.Effect of different concentrations of GA3 on pollen sterility and seed fertility



4.3.1.2.2. Peduncle length

The first three treatments viz., 100, 200 and 400 ppm GA_3 had no effect on peduncle length, but a sudden increase in peduncle length was noticed at 1600 ppm (24.3 cm) as compared to 20.5 cm recorded in control.

4.3.1.2.3. Flag leaf length and breadth

On comparison of the plants that received 100 and 200 ppm GA_3 to the plants that did not receive the same there was no effect of these two concentrations on flag leaf length. But a sudden elongation of flag leaf length was observed in plants that received higher concentrations of GA_3.

A reduction of 0.1 cm in breadth of flag leaf was recorded at all the three 100, 200 and 400 ppm of GA_3 concentration compared to control. However, a further reduction of 0.1 cm was recorded with each further increase in GA_3 concentrations.

4.3.1.2.4. Finger length

The lowest concentration of 100 ppm of the chemical had no effect on finger length. While 200 and 400 ppm GA_3 increased finger length to the same extent. A further increase in finger length at 800 ppm and 1600 ppm were recorded.

4.3.2. Effect of different concentrations of ethrel

4.3.2.1. Pollen sterility and seed fertility

Results are presented in Table 5. A look into the table depicts that all the treatments exhibited higher pollen sterility percentage than control. Though ethrel affected the pollen fertility at all doses, at lower doses the differences in fertility was not obvious. The chemical was more effective in inducing higher sterility at the apical and basal regions of the fingers than the middle region. There was no difference in sterility between apical and basal florets.

While 1000 ppm had negligible effect on seed fertility. However a further increase in concentration to 2000 ppm drastically affected seed fertility. Further increase in the concentrations reduced the seed fertility. Lowest seed set being recorded at the highest concentration. This is clearly depicted in Figure 4.

4.3.2.2. Morphological traits

Table 6 provides a detailed information on the results of different concentrations of ethrel on morphological traits.

Table 5. Effect of different concentrations of Etherel on pollen sterility and seed fertility of finger millet treated at 2-3 leaf stage

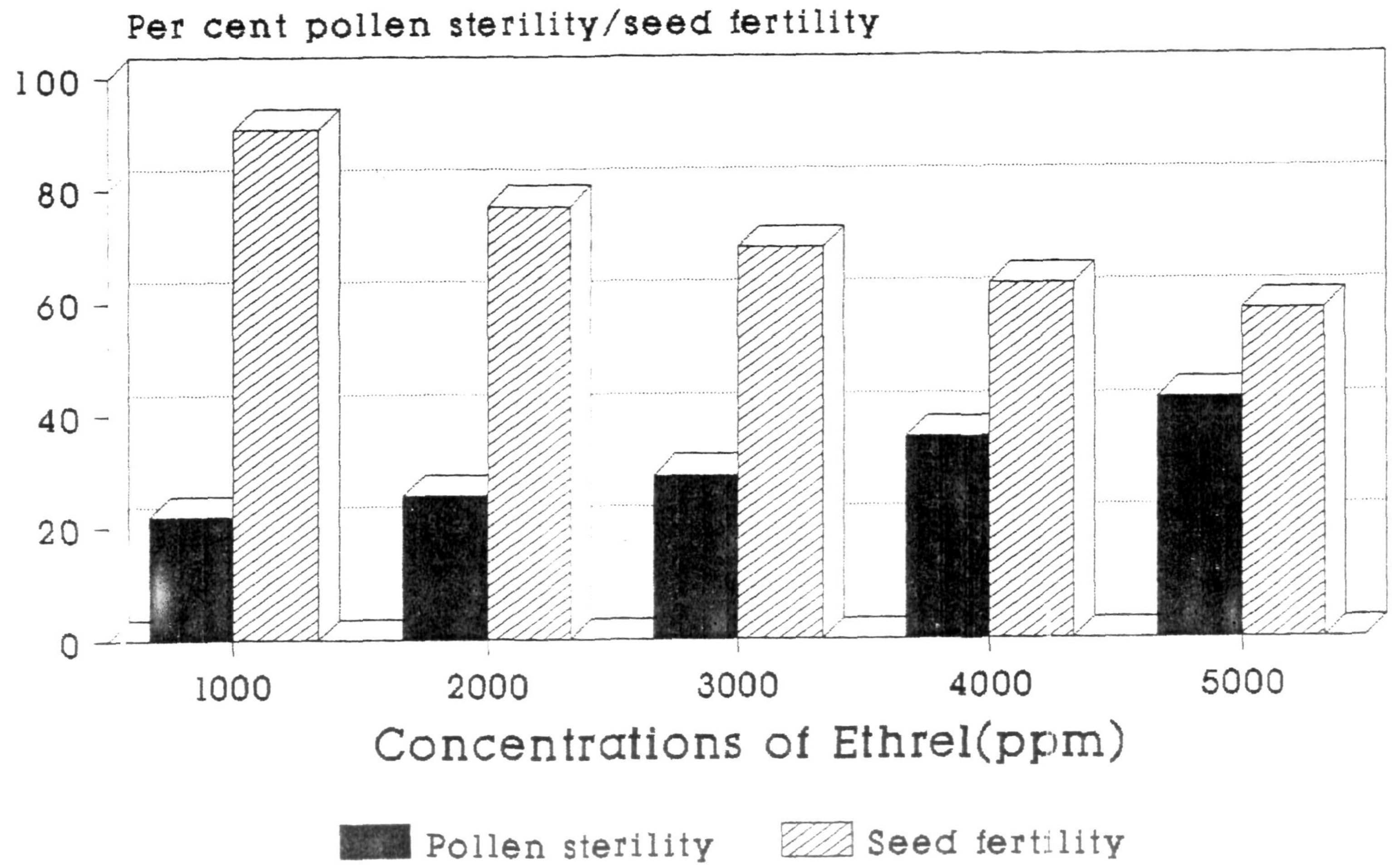
Concentrations of Ethrel	Mean pollen sterility						Mean pollen sterility	Mean seed fertility			Mean
	Spikelets at tip of the finger		Spikelets at middle of the finger		Spikelets at base of the finger			Spikelets at tip of the finger	Spikelets at middle of the finger	Spikelets at base of the finger	
	Apical floret	Basal floret	Apical floret	Basal floret	Apical floret	Basal floret					
1000 ppm	20.6	22.0	20.0	25.0	24.2	20.0	21.9	89.4	93.7	89.4	90.8
2000 ppm	25.0	26.9	24.2	21.1	31.1	25.0	25.5	72.2	82.3	76.5	77.0
3000 ppm	33.3	30.2	28.2	25.0	32.8	25.0	29.1	66.6	73.6	68.4	69.5
4000 ppm	38.9	35.0	35.0	34.0	36.6	35.5	35.8	61.1	63.2	64.7	63.0
5000 ppm	48.1	42.8	38.2	40.2	43.0	44.4	42.7	55.5	61.1	58.8	58.4
Control (No treatment)	7.5	7.3	7.1	6.9	7.4	7.3	7.3	99.5	100.0	99.0	99.5
Mean over apical and basal florets	32.3		29.1		31.7			68.9	74.5	71.5	

Mean of apical florets of tip, middle and base of finger = 31.9
Mean of basal florets of tip, middle and base of finger = 30.1

Table 6. Effect of different concentrations of Ethrel on morphological characters of finger millet

Concentration	Mean plant height (cm)	Mean peduncle length (cm)	Mean Flag leaf		Mean finger length (cm)
			Length (cm)	Breadth (cm)	
1000 ppm	109.0	13.4	25.6	1.4	6.1
2000 ppm	91.5	9.8	23.5	1.4	5.9
3000 ppm	86.6	8.7	22.7	1.6	5.8
4000 ppm	75.9	6.7	21.7	1.6	5.3
5000 ppm	61.7	6.5	21.4	1.6	4.7
Control (No treatment)	110.3	19.6	32.4	1.2	6.2

Fig.4.Effect of different concentrations of Ethrel on pollen sterility and seed sterility



4.3.2.2.1. Plant height

The lowest concentration of 1000 ppm did not affect the plant height but a considerable dwarfing of plant height could be noticed only at 2000 ppm and onwards. A severe dwarfing effect was noticed at the highest concentration of 5000 ppm.

4.3.2.2.2. Peduncle length

Though 1000 ppm ethrel had not affected plant height it did affect peduncle length by reducing it. With the increase in concentrations from 1000 ppm to 4000 ppm, peduncle length decreased but there was no reduction in height due to increase in concentration from 4000-5000 ppm.

4.3.2.2.3. Flag leaf length and breadth

From 32.4 cm flag leaf length of the normal plant, it got reduced to 25.6 cm even at the lowest concentration of 1000 ppm ethrel. Further reduction was recorded with the increase in concentration upto 300 ppm. Further increase in concentration upto a concentration of 5000 ppm did not affect the length.

1000 ppm of ethrel was effective in increasing breadth of flag leaf from 1.2 cm (control) to 1.4 cm. The similar increase in breadth was recorded at 2000 ppm also. While, the last three concentrations of 3000, 4000, 5000 ppm further increased the width to the same extent (1.6 cm).

Table 7. Effect of different concentrations of 2,4-D on pollen sterility and seed fertility in finger millet treated at 2-3 leaf stage

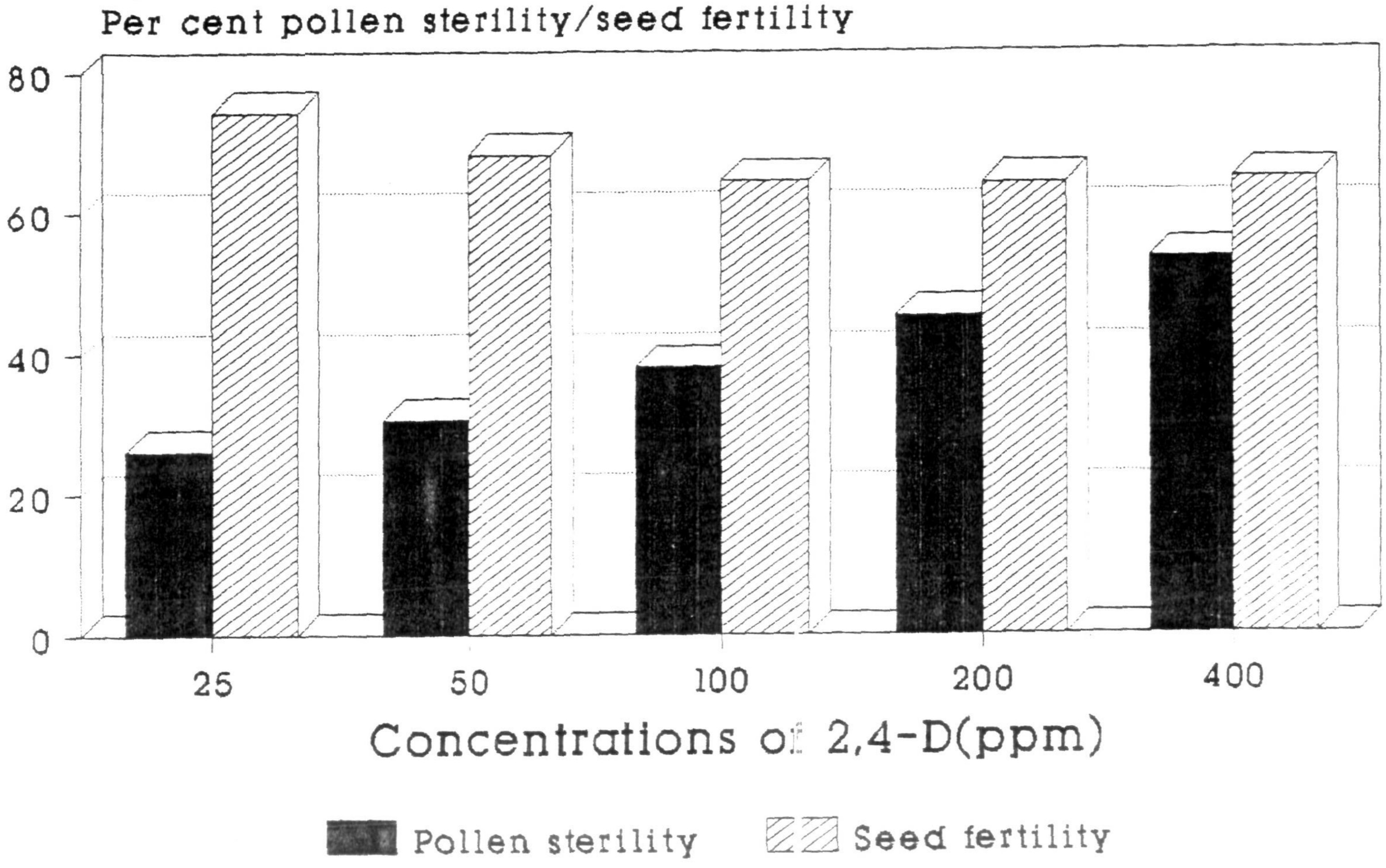
Concentrations of 2,4-D	Mean pollen sterility						Mean pollen sterility	Mean seed fertility			Mean
	Spikelets at tip of the finger		Spikelets at middle of the finger		Spikelets at base of the finger			Spikelets at tip of the finger	Spikelets at middle of the finger	Spikelets at base of the finger	
	Apical floret	Basal floret	Apical floret	Basal floret	Apical floret	Basal floret					
25 ppm	29.5	26.6	22.2	25.0	28.9	25.0	26.2	75.0	77.7	70.6	74.4
50 ppm	33.3	30.7	28.9	26.3	34.6	30.2	30.6	65.0	72.0	68.0	68.3
100 ppm	40.6	38.3	35.7	32.7	41.5	39.3	38.1	65.3	64.7	63.2	64.4
200 ppm	47.6	44.4	45.4	42.8	47.1	44.6	45.3	61.1	62.5	68.7	64.1
400 ppm	59.3	54.3	50.0	48.9	54.1	53.3	53.3	58.8	68.4	66.6	64.6
Control (without treatment)	4.5	5.0	5.5	4.9	5.3	5.0	5.1	98.5	98.0	99.7	
Mean over apical and basal florets	40.5		35.7		39.8			65.1	69.1	67.4	

Mean of apical florets of tip, middle and base of finger = 39.9
Mean of basal florets of tip, middle and base of finger = 37.5

Table 8. Effect of different concentrations of 2,4-D on morphological characters of finger millet

Concentration	Mean plant height (cm)	Mean peduncle length (cm)	Mean Flag leaf Length (cm)	Mean Flag leaf Breadth (cm)	Mean finger length (cm)
25 ppm	109.7	21.6	34.6	1.3	5.3
50 ppm	108.9	21.5	33.4	1.3	5.2
100 ppm	94.9	17.7	25.8	1.9	3.5
200 ppm	94.5	17.9	25.5	1.9	3.4
400 ppm	94.6	17.9	25.1	1.9	3.4
Control (No treatment)	115.8	22.3	33.5	1.1	6.4

Fig.5.Effect of different concentrations of 2,4-D on pollen sterility and seed fertility



4.3.3.2.1. Plant height

The lowest concentration of 25 ppm 2,4-D reduced plant height from 115.8 cm of control to 109.7 cm, but the next concentration of 50 cm did not further reduce the height while a sudden reduction was noticed at 100 ppm (94.9 cm) but the preceding concentrations did not reduce plant height.

4.3.3.2.2. Peduncle length

The first two concentrations of 25 and 50 ppm 2,4-D did not affect peduncle length as compared to control but from 50 to 100 ppm there was a sudden reduction in peduncle length whereas beyond this concentrations the length was not affected.

4.3.3.2.3. Flag leaf length and breadth

The length of flag leaf was not affected by 25 and 50 ppm 2,4-D as against normal plant while a sudden reduction in length was noticed at 100 ppm but further reduction was not noticed at 200 and 400 ppm.

The two lowest concentrations of 25 and 50 ppm increased width of the flag leaf by 0.2 cm, while it still increased by 0.7 cm in all the concentrations of 100 ppm, 200 and 400 ppm as compared to control (1.1. cm).

4.3.3.2.4. Finger length

25 and 50 ppm 2,4-D reduced finger length to the same amount, while 100 ppm drastically reduced the finger length, but 200 and 400 ppm did not show further reduction in finger length.

4.4. EXPERIMENT IV: EFFECT OF 1600 PPM GA_3 AND 100 PPM 2,4-D APPLIED AT FIVE DIFFERENT GROWTH STAGES OF FINGER MILLET

4.4.1. Effect of 1600 ppm GA_3

4.4.1.1. Pollen sterility and seed fertility

It is seen from the Table 9, that GA_3 treatment at all leaf stages has increased sterility as compared to no treatment. The pollen sterility increased with the application of GA_3 from late growth stage (1st leaf stage) to the early growth stage (fifth leaf stage) (Fig.6). The highest mean sterility of 62.4 per cent was recorded when GA_3 was applied at fifth leaf stage. Among the three different positions of spikelets affected tip was affected to a greater extent followed by middle finger and basal finger. Between the apical and basal florets apical florets showed higher sterility of 37.3 per cent compared to 35.8 per cent recorded by basal florets.

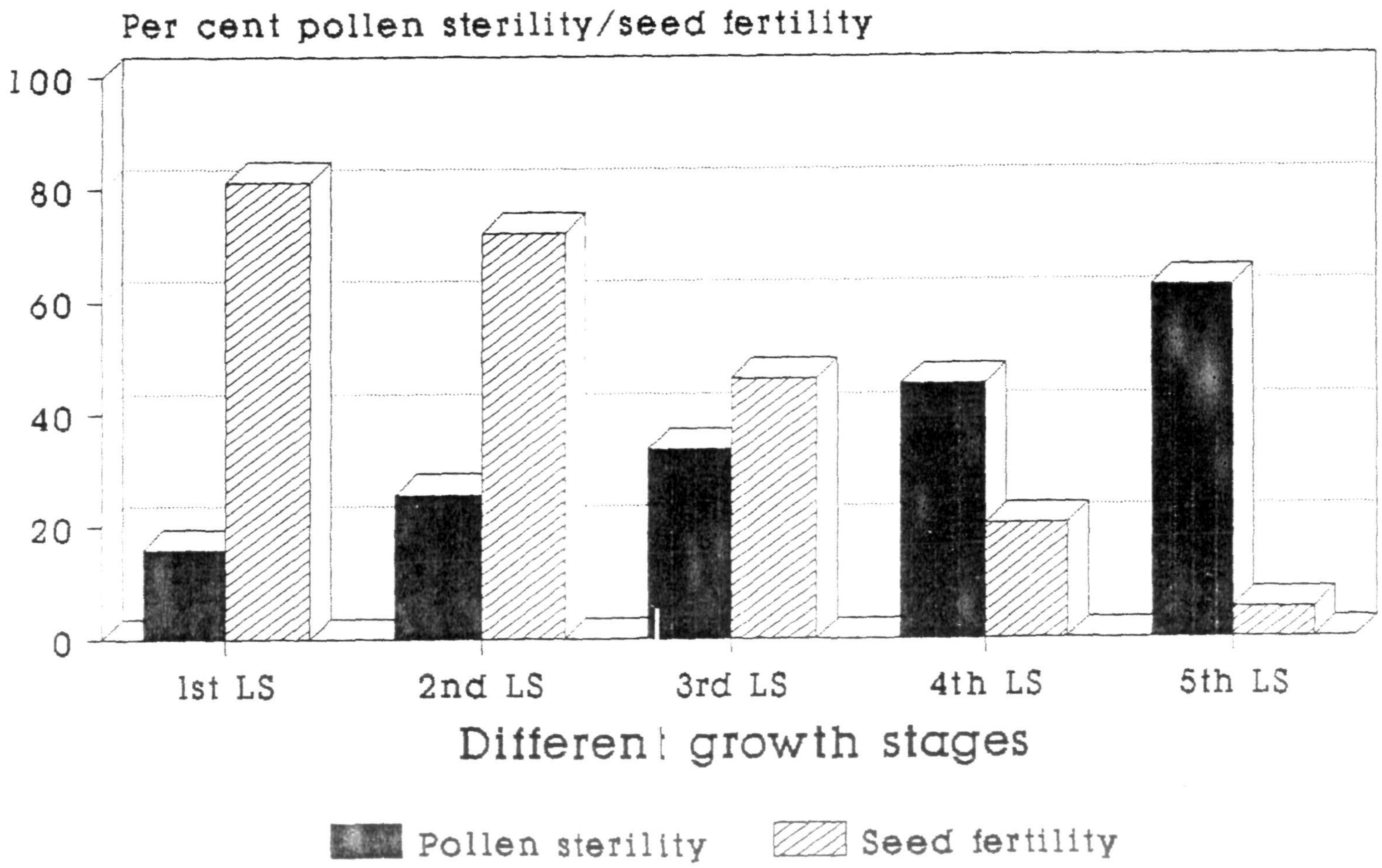
With regard to seed fertility spraying at later growth stages (first leaf stage) recorded slightly lower fertility (81.3%) as compared to control

Table 9. Effect of 1600 ppm GA_3 on pollen sterilit and seed fertility of finger millet ears treated at five different leaf stages

Different growth stages	Mean pollen sterility						Range	Mean pollen sterility	Mean seed fertility			Range	Mean
	Spikelets at tip of the finger		Spikelets at middle of the finger		Spikelets at base of the finger				Spikelets at tip of the finger	Spikelets at middle of the finger	Spikelets at base of the finger		
	Apical floret	Basal floret	Apical floret	Basal floret	Apical floret	Basal floret							
1st Leaf stage	14.5	22.5	12.2	14.9	13.9	16.9	0.0-27.3	15.8	82.4	86.4	75.0	66.6-92.3	81.3
2nd Leaf stage	26.2	23.5	30.7	35.9	16.2	21.7	0.0-100.0	25.7	68.4	75.0	72.7	63.6-76.9	72.1
3rd Leaf stage	48.3	39.1	27.4	30.4	30.4	27.7	12.5-51.7	33.8	47.4	55.0	36.0	30.0-58.3	46.2
4th Leaf stage	46.0	40.0	48.3	32.9	49.2	54.2	23.1-80.0	45.1	11.1	26.3	23.5	16.6-36.4	20.3
5th Leaf stage	69.8	69.5	70.4	58.3	55.5	50.9	11.7-92.3	62.4	5.3	5.0	5.0	0.0-9.1	5.1
Control (without treatment)	6.3	6.2	6.0	6.5	5.9	5.4		6.1	95.0	98.0	95.3		96.1
Mean over apical and basal florets	39.9		36.14		33.6				42.9	49.5	42.4		

Mean of apical florets of tip. middle and base of finger = 37.3
Mean of basal florets of tip. middle and base of finger = 35.8

Fig.6. Effect of 1600 ppm GA3 on pollen sterility and seed fertility treated at five different leaf stages



(96.1%). Among the stages of treatment application of GA_3 at early growth stage recorded the least of 5.1 per cent fertility and the fertility increased with the delayed application.

Range of pollen sterility was the highest at second leaf stage of 100 per cent but the range of seed fertility was highest at first leaf stage of 92.3 per cent.

4.4.1.2. Morphological traits

Effect of GA_3 application on morphological traits is presented in Table 10.

4.4.1.2.1. Plant height

It is observed from the table that application of GA_3 at different growth stages affected the plant height. The plant height increased to 67.9 cm from 56.5 cm (control) when the application was made at first leaf stage. Application at fifth leaf stage almost doubled the plant height from 56.5 cm (control) to 109.3 cm (fifth leaf stage).

4.4.1.2.2. Peduncle length

The peduncle length was not affected much when the GA application was made at first and second leaf stage. However, peduncle length increased

Table 10. Effect of 1600 ppm GA_3 on morphological characters of finger millet ears

Stage of treatment	Mean plant height (cm)	Mean peduncle length (cm)	Mean Flag leaf Length (cm)	Mean Flag leaf Breadth (cm)	Mean finger length (cm)
1st Leaf stage	67.9	21.6	24.0	2.7	4.3
2nd Leaf stage	76.6	24.6	30.6	2.7	6.3
3rd Leaf stage	83.6	28.4	32.6	2.3	8.8
4th Leaf stage	86.6	31.0	40.6	1.8	14.5
5th Leaf stage	109.3	38.4	50.3	1.4	16.8
Control (No treatment)	56.5	22.5	30.6	2.0	4.3

Plate 2 Effect of 1600 ppm GA_3 on finger and glume elongation applied at first leaf stage

Plate 3 Effect of 1600 ppm GA_3 on finger and glume elongation applied at second leaf stage

Plate 4 Effect of 1600 ppm GA_3 on finger and glume elongation applied at third leaf stage

Plate 5 Effect of 1600 ppm GA_3 on finger and glume elongation applied at fourth leaf stage

Plate 6 Effect of 1600 ppm GA_3 on finger and glume elongation applied at fifth leaf stage

Plate 7 Glumes, lemma, palea, intact ovary, style and anthers of untreated plant

Plate 8 Effect of 1600 ppm GA_3 on elongation of glumes, lemma, palea, ovary, style and anthers when applied at second leaf stage

Plate 9 Effect of 1600 ppm GA_3 on elongation of glumes, lemma, palea, ovary, style and anthers when applied at third leaf stage

Plate 10 Effect of 1600 ppm GA_3 on elongation of glumes, lemma, palea, ovary, style and anthers when applied at fourth leaf stage

Plate 11 Effect of 1600 ppm GA_3 on elongation of glumes, lemma, palea, ovary, style and anthers when applied at fifth leaf stage

from 22.5 cm to 28.4 cm due to application of GA_3 at third leaf stage. The longest peduncle length of 38.4 cm was recorded when the application of GA_3 was made at fifth leaf stage.

4.4.1.2.3. Flag leaf length and breadth

As regards to flag leaf length and breadth, there was no effect of length and breadth of flag leaf when the GA was applied at first and second leaf stage, in contrast application at third leaf stage increased the flag leaf length but the width was reduced. The longest and the narrow flag leaf was recorded when GA was applied at fifth leaf stage.

4.4.1.2.4. Finger length

Application of GA_3 at first leaf stage did not affect the finger length however, application at second and third leaf stage marginally increased finger length with substantial increase of finger length from 4.3 cm to 14.5 cm was recorded with the application at fourth leaf stage. Advancing the application at fifth leaf stage increased the finger length marginally (Plate 2 to 6).

There was a stead elongation in the length of glumes, lemma, plaea, ovary and style from second to fifth leaf stages (Plate 8 to 11).

4.4.2. Effect of 100 ppm 2,4-D

4.4.2.1. Pollen sterility

The effect of 100 ppm 2,4-D on pollen and seed fertility is presented in Table 11. Irrespective of the leaf stages application of 2,4-D has considerably increased the pollen sterility. Application of 2,4-D at later growth stages (first leaf stage) has recorded a mean sterility of 24.8 per cent. Further preparing application to second leaf stage did not affect the sterility. However, the sterility increased progressively when the application was prepared from third leaf to fifth leaf stage. The fifth leaf stage application has recorded the maximum sterility of 53.5 per cent as compared to 6.2 per cent sterility in control. The tip spikelets showed higher sterility compared to middle and base spikelets. Not much difference in sterility between apical and basal florets was noticed.

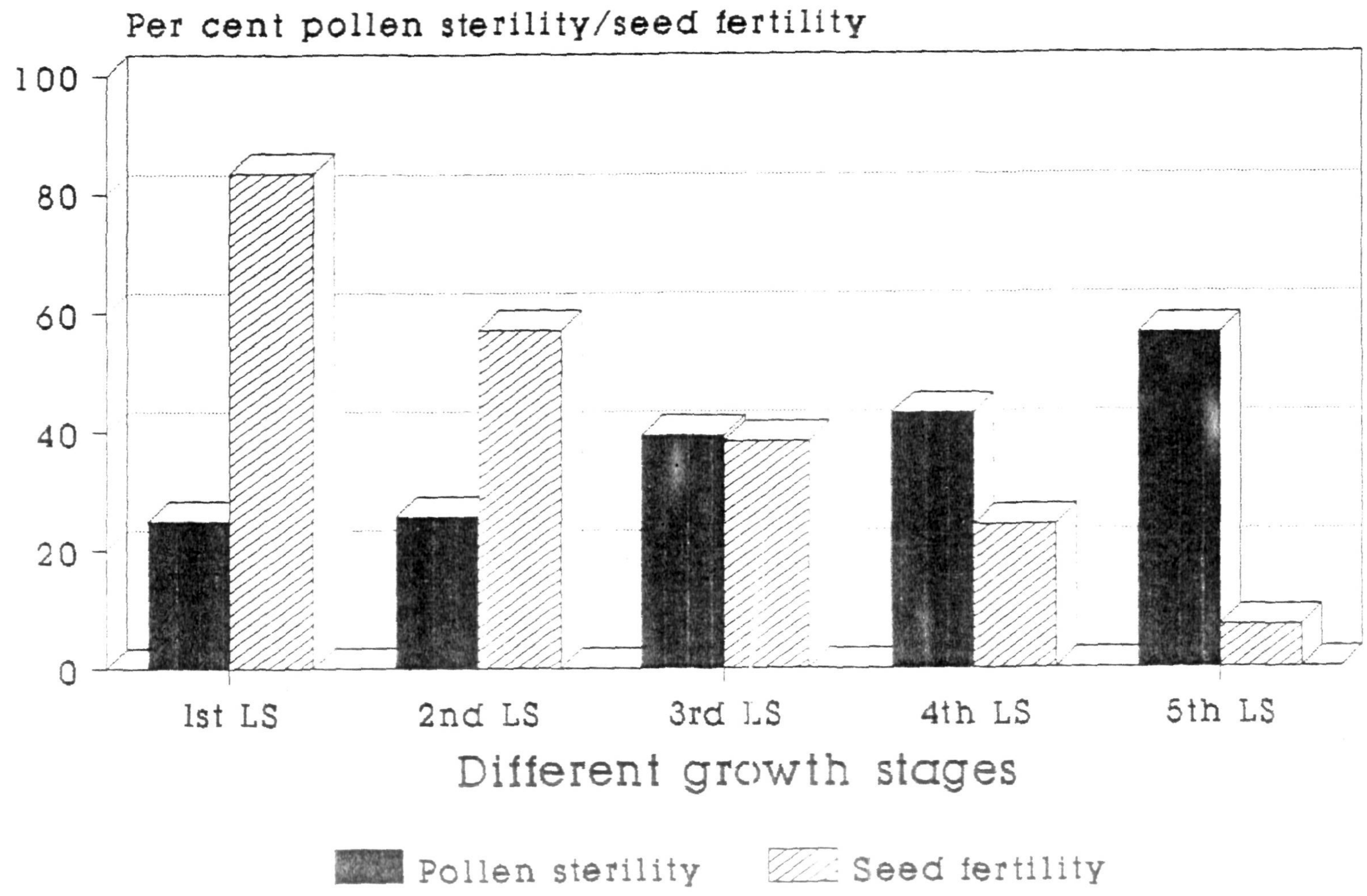
Application of 2,4-D at later growth stage (first leaf stage) marginally decreased the fertility from 98.5 per cent in control to 83.6 per cent recorded in first leaf stage. A sudden reduction in fertility from 83.6 per cent to 57.0 per cent was recorded when the chemical was applied at second leaf stage. The least fertility of 7.1 per cent was recorded when the application was made at fifth leaf stage (early growth stage) (Fig.7). Apical spikelets (43.7) recorded more sterility followed by middle (44.1) and basal spikelets (38.2).

Table 11. Effect of 100 ppm 2,4-D on pollen sterility and seed fertility of finger millet

Different growth stages	Mean pollen sterility: Spikelets at tip of the finger – Apical floret	Basal floret	Spikelets at middle of the finger – Apical floret	Basal floret	Spikelets at base of the finger – Apical floret	Basal floret	Range	Mean pollen sterility	Mean seed fertility: Spikelets at tip of the finger	Spikelets at middle of the finger	Spikelets at base of the finger	Range	Mean
1st Leaf stage	28.1	24.6	25.2	33.3	22.5	15.2	9.1-5.0	24.8	84.2	83.3	83.3	72.7-100.0	83.6
2nd Leaf stage	36.4	37.2	21.1	16.6	27.3	14.8	0.0-52.5	25.5	64.7	56.3	50.0	36.4-84.6	57.0
3rd Leaf stage	43.3	36.9	39.8	31.6	42.3	40.9	10.5-75.0	39.2	42.1	42.8	30.0	16.6-60.0	38.3
4th Leaf stage	46.9	40.8	39.3	42.8	45.3	41.8	17.8-69.5	42.8	22.2	27.7	22.2	8.3-41.6	24.1
5th Leaf stage	52.1	66.6	54.4	59.3	52.8	52.6	33.3-75.0	56.3	5.3	10.5	5.3	0.0-8.0	7.1
Control (without treatment)	6.5	6.3	6.0	5.9	6.3	6.4		6.2	99.0	98.6	99.0		98.8
Mean over apical and basal florets	41.3		36.3		35.5				43.7	44.1	38.2		

Mean of apical florets of tip, middle and base of finger = 38.4
Mean of basal florets of tip, middle and base of finger = 37.0

Fig.7. Effect of 100 ppm 2,4-D on pollen sterility and seed fertility



Highest pollen sterility range was recorded at third leaf stage while highest seed fertility range was recorded at first leaf stage.

4.4.2.2. Morphological traits

Effect of 100 ppm 2,4-D on morphological traits is presented in Table 12.

4.4.2.2.1. Plant height

2,4-D reduced the plant height when applied at different growth stages. Hundred per cent reduction in height from 66.5 cm to 33.6 cm was recorded when the application of 2,4-D was made at early growth stage (fifth leaf stage).

4.4.2.2.2. Peduncle length

A similar trend as observed in plant height was also recorded for the character peduncle length. Though there was no reduction in peduncle length when the application was made at first leaf stage considerable reduction in length was recorded with the application of 2,4-D from third leaf stage onwards. Application at fifth leaf stage recorded the shortest peduncle of 10.2 cm compared to 26.5 cm recorded on untreated plants.

Table 12. Effect of 100 ppm 2,4-D on morphological traits

Stage of treatment	Mean plant height (cm)	Mean peduncle length (cm)	Mean Flag leaf Length (cm)	Mean Flag leaf Breadth (cm)	Mean finger length (cm)
1st Leaf stage	58.2	26.1	36.4	1.2	5.9
2nd Leaf stage	52.1	19.3	34.5	1.2	4.9
3rd Leaf stage	45.4	16.3	33.1	1.4	3.9
4th Leaf stage	39.3	12.1	30.2	1.8	3.4
5th Leaf stage	33.6	10.2	28.4	1.8	2.4
Control (No treatment)	66.5	26.5	40.3	1.0	6.0

Plate 12 Effect of 100 ppm 2,4-D on finger and glume length reduction applied at first leaf stage

Plate 13 Effect of 100 ppm 2,4-D on finger and glume length reduction applied at second leaf stage

Plate 14 Effect of 100 ppm 2,4-D on finger and glume length reduction applied at third leaf stage

Plate 15 Effect of 100 ppm 2,4-D on finger and glume length reduction applied at fourth leaf stage

Plate 16 Effect of 100 ppm 2,4-D on finger and glume length reduction applied at fifth leaf stage

Plate 17 Effect of 100 ppm 2,4-D in reducing the iengths of glume, lemma, palea, ovary, style and anthers applied at second leaf stage

Plate 18 Effect of 100 ppm 2,4-D in reducing the lengths of glume, lemma, palea, ovary, style and anthers applied at third leaf stage

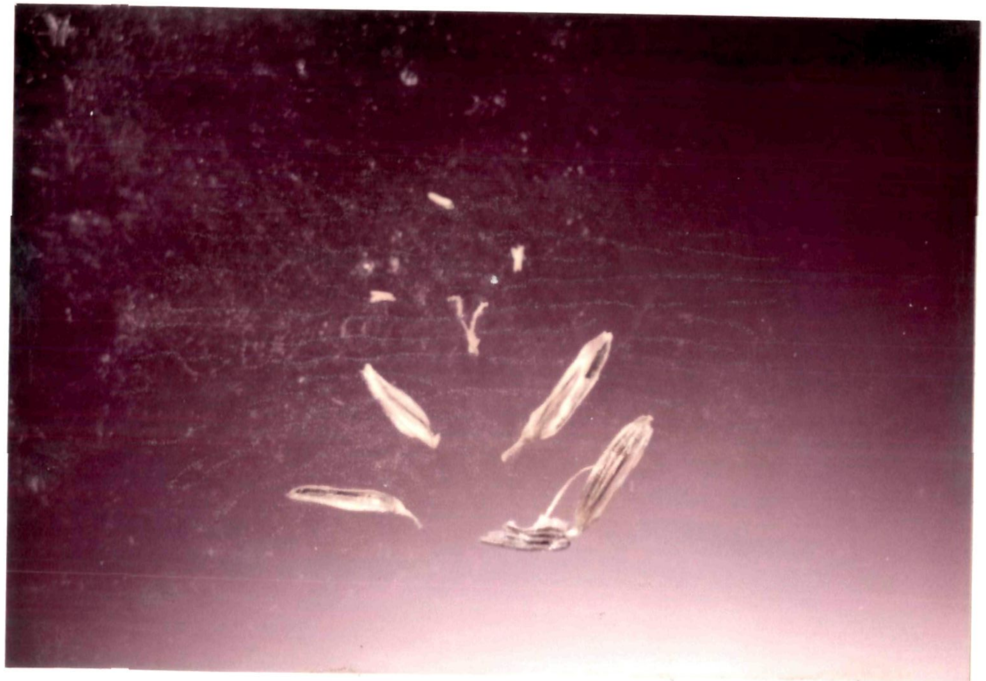


Plate 19 Effect of 100 ppm 2,4-D in reducing the lengths of glume, lemma, palea, ovary, style and anthers applied at fourth leaf stage

Plate 20 Effect of 100 ppm 2,4-D in reducing the lengths of glume, lemma, palea, ovary, style and anthers applied at fifth leaf stage

4.4.2.2.3. Flag length and breadth

Reduction of flag leaf length and increase in breadth was recorded due to application of 2,4-D at different growth stages. The shortest and broadest flag leaf was recorded when 2,4-D was applied at earl growth stage ie., when the junctions of all the five leaves were invisible (early growth stage).

4.4.2.2.4. Finger length

Application of 2,4-D at later growth stage (first leaf stage) did not affect the finger length, in contrast application at second to fifth leaf stage decreased the finger length substantially. The shortest finger of 2.4 cm was recorded when 2,4-D was applied at early growth stage as compared to 6 cm length of untreated check plant (Plate 12 to 16).

There was a steady reduction in the length of glumes, lemma, palea, ovary and style from second to fifth leaf stages (Plate 17 to 20).

DISCUSSION

V. DISCUSSION

Over the past four decades ragi production has risen substantially. Production has been improved mainly through the varietal component of production. Till 1970's, the genetic improvement in this crop was mainly through pure line selection. However, in 1970s hybridization was initiated in a limited scale. Substantial yield improvement was possible even with limited hybridization. At present, there is a plateau in varietal improvement even though a wide range of genetic resources have been accumulated in this crop. Not even one per cent of the available germplasm could be utilised so far mainly due to non availability of a simple hybridization technique. High volume crossing utilising the diverse germplasm seems to be the only answer for breaking the yield plateau. A survey of the available emasculation and crossing techniques indicated that only contact method of hybridization is being practiced. The reasons for not following the other technique followed in other crops are: (1) Ragi is predominantly an autogamous crop with very small florets (2) Anthesis takes place in the very early hours of the day and (3) the pollen is viable for a short period. Under these circumstances a simple technique to induce male sterility will be of immense practical significance. Though hot water emasculation and use of gametocides were found to induce male sterility, no systematic study was initiated to standardize the technique for large scale adoption in finger millet.

In order to generate information and to standardize the technique of hot water and chemical emasculation, experiments were conducted and results obtained are discussed and the feasible technology for emasculation in ragi is suggested.

5.1. HOT WATER EMASCULATION

5.1.1. Appropriate water temperature and period of treatment

The temperature of 45 to 50°C for a standard duration of five minutes was chosen in the experiment to identify the most effective temperature based on previous literatures of Rama Rao and Rama Rao (1962), Srivastava and Yadav (1972) and Raj *et al.* (1964). The results of the first experiment have brought out that the temperature range of 48-49°C was the most effective in inducing maximum pollen sterility and the minimum seed set than others. Therefore the temperature range of 48-49°C was selected for second experiment. For obtaining further increase in sterility and low seed set a range of immersion durations from 3.0 minutes to 9.0 minutes were included. The results of the second experiment had clearly brought out that immersion of ragi ears in 48-49°C hot water for a duration of 6.0 minutes to be effective in inducing maximum pollen sterility and low seed set. Further increase of immersion period beyond 6.0 minutes resulted in drying up of ears.

The range of pollen fertility has indicated that even immersion for 3 minutes could induce 100 per cent sterility in a few florets. However, the frequency of such florets was high when the ear was immersed for 6 minutes. The high sterility in some florets even at lower exposure period could be due to their being in optimum stage at the time of immersion compared to pollen fertility, the seed fertility was very low. This suggests that though the pollen looks apparently fertile, it might have losts its viability. The available literature suggests that anthesis within a finger starts at two thirds tip portion of the finger and proceeds towards apex and base indicating that microsporogenesis first begins at this region and proceeds in either directions as a result quick extrusion of anthers or anthesis is observed at this region followed by tip and basal regions. Based on this aspect and the fact that highest pollen sterility was recorded at the base of the finger with the lowest amount of seed set instead of immersing the entire ear the spikelets at the base of the finger could be retained for hot water treatment. After the treatment the treated fingers can then be pollinated by the desired male parent to get the highest percentage of hybrid seeds.

5.2. EMASCULATION USING GAMETOCIDES

5.2.1. Selection of Gametocides

Among the three gametocides tried namely gibberellic acid, ethrel and 2,4-D only two chemicals viz., gibberellic acid and 2,4-D appeared promising since the highest pollen sterility was obtained with these chemicals as compared to ethrel. Hence out of the three chemicals only gibberellic acid and 2,4-D were selected. The results of these two chemicals as a male gametocide are discussed.

5.2.2. Gibberellic acid as a male gametocide for ragi

When GA_3 was applied at 2-3 leaf stage, with the increase in concentration from 100 to 1600 ppm there was an increase in pollen sterility and a steady decrease in seed set. The highest concentration of 1600 ppm induced the highest percentage of pollen sterility and recorded the lowest seed set. Hence the concentration of 1600 ppm GA_3 was chosen. Though arbitrarily 2-3 leaf stage was fixed for the application of the chemical to get better results earlier and later stages of growth were included in the fourth experiment. As such 1st, 2nd, 3rd, 4th and 5th leaf stages of growth periods were included in the final experiment. Inclusion of these growth periods was based on the literatures of Schuster (1969) who treated sunflower plants at the beginning of bud development with 0.25 to 0.50 mg per plant of GA_3 and observed 90 to 100 per cent of male sterility, Borghi and Pironi (1970) who reported that two applications of ethrel at 1 or 2 and 3 to 4 true leaf stages at concentrations of 600 to 800 ppm in *Cucurbita pepo* and 500 to 600 ppm in cucumber caused male sterility. Law and Stoskopf (1973) also reported 96 per cent male sterility in barley plants treated with ethrel at the time of panicle primordial development stage and under field conditions male sterility upto

55 per cent was induced when 1.4 kg per ha of ethrel was applied at the flag leaf emergence stage. Perez *et al.* (1973) found that spraying of ethrel at the early booting stage induced high pollen and spikelet sterility in wheat while Tarakanov and Ayapova (1973) reported a shift towards female sex in cucumber when treated with ethrel at two leaf stage. Borghi *et al.* (1974) observed complete sterility when Triticum aestivum varieties Flamingo and S Pastore and *Triticum durum* variety lambo were treated with 2000 ppm of 2-chloro ethare phosphonic acid spray at early, mid, late booting and early heading stages. Seetharam and Kusumakumari (1974) reported that application of GA_3 on the 3rd day after flower initiation induced maximum pollen sterility in sunflower and the extent of pollen sterility decreased as the time of treatment was delayed while Spirova (1975) successfully induced male sterility in four inbred lines of sunflower when 0.5 mg GA was applied at 5 to 6 leaf stage and at the flower bud stage. Hughes *et al.* (1978) reported the application of GA_3 at the rate of 12.8 kg a.i. per ha in combination with 6.4 kg ethephon a.i. per ha before meiosis induced maximum male sterility.

The results of the present study has clearly indicated that there was a progressive increase in sterility as the treatment was advanced. Treatment at early growth stage (5th leaf stage) which is 14 days before emergence of panicle from boot leaf has resulted in the highest percentage of pollen sterility and lowest seed set. Thus it could be concluded that GA_3 could be effectively used to induce male sterility at 1600 ppm and applied 14 days before the emergence of panicle from the boot leaf. Similar results have also been obtained by Nelson and Rossman (1958) in maize, Bose and Nitsch (1970) in *Luffa acutangula*, in sunflower by Klimov (1973) and Seetharam and Kusumakumari (1974).

Gibberellic acid not only affected pollen sterility and seed set, it also had an effect on other traits. It increased plant height, peduncle length, flag leaf length and finger length while the flag leaf breadth was reduced. Such an effect of GA_3 on morphological traits was noticed by Singh *et al.* (1980) and Vadivelu *et al.* (1984) in sorghum, Rajni *et al.* (1992) and Thangaraj and Sivasubramaniam (1992) in rice while in pea it was noticed by Podesva and Sebanek (1986).

5.2.3. 2,4-D as male gametocide on ragi

Third experiment involved the application of 25, 50, 100, 200 and 400 ppm 2,4-D at 2-3 leaf growth stage. The results of the third experiment indicated low seed set at 100, 200 and 400 ppm though the increase in trend of pollen sterility was noticed with increase in concentration of the chemical from 100 to 400 ppm. Hence 100 ppm 2,4-D was selected and applied at five growth stages of ragi crop. The results of the fourth experiment suggested that 2,4-D applied at fifth leaf stage induced maximum pollen sterility (56.3%) and least seed fertility (7%). It was also noticed that 2,4-D adversely affected plant height, peduncle length, flag leaf length and finger length while it increased the breadth of flag leaf. Results similar to the present study are also obtained by Lyaskovski (1990) and Yaduraj and Ahuja (1992) in wheat, while in barley and *Lepidium sativum* it was noticed by Podesva and Sebanek (1986) and Patil *et al.* (1992) noticed it in cotton.

5.3. FEASIBLE TECHNOLOGY

Of the three experiments involving hot water, gibberellic acid and 2,4-D it was found that hot water emasculation seems to be the most appropriate and feasible technology besides being cheap and simple. Hence, the technique and procedure involved in the hot water method of emasculation is prescribed below.

5.3.1. Methodology for hot water emasculation

Materials required: A wide mouthed thermous flask with a capacity to hold one litre of water, a thermometer that can measure upto sixty degree celicius, hot water of 60°C, cold water, a thermocool lid with a hole to insert the thermometer and a slit made from the edge of the lid so as to hold the peduncle of the panicle. The thermocool lid should fit exactly into the mouth of the flask.

Procedure:

1. The ears are prepared by retaining the spikelets at the base of the finger and removing the upper and middle portions.

Insert the peduncle of the prepared ear and thermometer inversely into the thermocool lid prepared before.

2. Pour the hot water of 60°C to the thermosflask. Add cold water to bring down the temperature to the range of 48-49°C.

3. Immediately cover the lid possessing thermometer and ear on to the mouth of thermosflask and allow the ear and thermometer for 6.0 minutes inside hot water of 48-49°C. Remove the ear from hot water immediately after 6.0 minutes of immersion

4. Contact the treated ear with the desired male parent for effective hybridization.

SUMMARY

VI. SUMMARY

The present investigation was taken up with hot water and three hormones viz., (1) Gibberellic acid (2) Ethrel and (3) 2,4-D with the objective of (1) identification of the most appropriate hot water temperature and time of immersion required to induce male sterility and (2) select the ideal gametocide and the stage of application to get high male sterility. The cultivar used in the study was PR.202. Hot water temperature ranges from 45 to 50°C for 5 minutes and the range of 48 to 49°C for 3.0 to 9.5 minutes was used. The three chemicals viz., GA_3 with 100, 200, 400, 800 and 1600 ppm concentrations, Ethrel with 1000, 2000, 3000, 4000 and 5000 ppm concentrations and 2,4-D with 25, 50, 100, 200 and 400 ppm concentrations were used. The study was conducted at the Main Research Station, Gandhi Krishi Vignana Kendra, University of Agricultural Sciences, Bangalore. The salient features of research findings are summarised below.

1. 1st, 2nd, 3rd, 4th and 5th leaf stages appears 6, 8, 10, 13 and 15 days prior to the emergence of panicle from boot leaf.

2. Hot water emasculation: Increase in hot water from 45-46°C to 48-49°C increased pollen sterility and decreased seed set steadily. Further increase in temperature of hot water had a deleterious effect resulting in drying up of the ears. The highest mean pollen sterility of 67.9 per cent and the lowest seed set of 6.6 per cent was recorded at 48-49°C of hot water with an

immersion time of 5 minutes. Further increase in immersion time by one minute has further increased the mean pollen sterility to 82.0 per cent but the grain set decreased marginally to 6.1 per cent.

3. Use of Gametocides: Of the three gametocides, GA_3 and 2,4-D were most effective in inducing male sterility as compared to ethrel. GA_3 recorded the highest pollen sterility of 46.9 with the lowest seed set per cent of 45.5 per cent at 1600 ppm. In contrast, 2,4-D had given 53.5 per cent pollen sterility with 64.6 per cent seed set at 400 ppm as compared to 42.7 per cent pollen sterility and 58.4 per cent seed set of ethrel. With the preponement in the stage of application of GA_3 the mean pollen sterility increased and seed set decreased. The highest pollen sterility of 62.4 per cent and the lowest seed set of 5.1 per cent was obtained when the application of GA_3 at 1600 ppm was made when the crop was in the 5th leaf stage ie., 62 days after sowing. Similar results with regard to 2,4-D was also obtained. 2,4-D concentration of 100 ppm applied at 5th leaf stage recorded 56.3 per cent pollen sterility and 7.1 per cent seed set.

The growth hormones effected the morphological traits also. GA_3 affected all the traits viz., plant height, peduncle length, flag leaf length, finger length were increased while it reduced breadth of flag leaf. But 2,4-D reduced plant height, peduncle length, flag leaf length, finger length and increased flag leaf breadth.

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***Original not seen.**

Appendix I. Days taken for complete emergence of panicle from the date of sowing

Plant Nos.	No.of days taken for complete emergence of panicle from the date of sowing
1	75
2	77
3	78
4	78
5	77
6	75
7	78
8	77
9	77
10	77
11	75
12	76
13	75
14	75
15	76
16	76
17	76
18	77
19	78
20	78
21	76
22	76
23	78
24	77
25	75
Mean	76.6

Appendix II. Identification of growth stages: Days taken for ear energence from boot leaf from first to fifth stage

Growth stage and Plant Nos.	No. of days	Mean	Average No. of days required to attain the stage	No. of days before panicle emerges from flag leaf
1st Leaf stage(flag leaf junctura is invisible)				
1	6			
2	6			
3	6	5.8	70.8	6
4	5			
5	6			
2nd Leaf stage(juncturas flag leaf alongwith one preceding leaf invisible)				
1	9			
2	9			
3	9	9.2	68.4	8
4	9			
5	10			
3rd Leaf stage(junctura of flag leaf alongwith two preceding leaves invisible)				
1	11			
2	11			
3	11	11.6	66.0	10
4	12			
5	12			
4th Leaf stage(junctura of flag leaf alongwith three preceding leaves invisible)				
1	14			
2	13			
3	14	13.8	63.8	13
4	14			
5	14			
5th Leaf stage(junctura of flag leaf alongwith four preceding leaves invisible)				
1	15			
2	16			
3	16	15.6	62.0	15
4	15			
5	16			