International Journal of Environment and Climate Change

Volume 14, Issue 2, Page 979-992, 2024; Article no.IJECC.108950
ISSN: 2581-8627
(Past name: British Journal of Environment & Climate Change, Past ISSN: 2231–4784)

Identification of Male Sterile Lines and Floral Characterization of White Onion (*Alium cepa)*

Naveenkumar B S [a*], Veere Gowda R [b++], Padmini K [b++] and Rajshree Gayen [a#]

[a] IGKV-Raipur, India.
[b] ICAR-IIHR. Bengaluru, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJECC/2024/v14i24011

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/108950

Original Research Article

Received: 20/09/2023
Accepted: 25/11/2023
Published: 28/02/2024

ABSTRACT

The investigation was conducted at the Division of Vegetable Crops, IIHR- Bengaluru. The experiment was carried out with a Randomized block design with 10 treatments and 3 replications namely PW0-18, TW-18, PWR-18, RW-18, and AW-O-18 these Male sterile lines, and their respective fertile lines were identified by visual observation, staining, and bagging techniques.

Keywords: Onion; staining; fertile; sterile; bagging.

[++] Principal Scientist;
[#] Associate Professor;
**Corresponding author: E-mail: naveenkumar7148@gmail.com;*

1. INTRODUCTION

Onion is the commercial vegetable of India with huge foreign exchange earnings (Over 1443.09 crores). Genetic improvement of onion will positively affect the national economy. Jones and Mann [1] have suggested Pakistan, Iran, and the mountainous areas of north India as their origin. India has an area of 12.30 Lakh ha in the world with a production of 215.64 lakh tons and a productivity of 16.16t/ha [2,3-9]. The leading producer of onion is Maharastra followed by Karnataka and Madhya Pradesh. India stands third in exports [10,11-17].

Each umbel contains flowers with yellow-coloured anthers in fertile followed by light pink coloured pollens in sterile and contains 6 stamens, 3 carpels united into the pistil and 6 perianth segments [18-23]. The pistil has 3 locules each of which has 2 ovules. Anthesis occurs early at the morning at 6-7 am and anther dehiscence continues after anthesis at 7 am to 5 pm [24-28]. Yellow anthers have a greater number of pollens we can observe followed by a smaller number of pollens in the green anthers comparatively.

2. MATERIALS AND METHODS

2.1 Nursery Preparation

The crop was raised under irrigation in sandy loom soil. The nursery beds of size. 5 x 0.75m having 15 cm height were prepared and a sufficient amount of farmyard manure was incorporated into the soil. The seeds were sown thinly in three rows of each line with spacing five cm between the lines. Seeds were covered with fine organic manure. The beds were covered with dry grass and water thoroughly. The dry grass was removed after the complete germination of seeds. The seedlings were ready for transplanting after forty-five days.

2.2 Preparation of Main Field

The experimental field is made into fine tilth by repeated plowing, closing, and removing weeds from the field. Farmyard manure was incorporated into the soil at the rate of 25 tonnes per hectare and the land was perfectly leveled. The uniform plots of size 2m x 1.0m were prepared. Fertilizers at the rate of 75 kg of nitrogen, 35 kg of P_20_5 and 50 kg of K_20 per hectare were applied in the form of ammonium sulphate single superphosphate and muriate of potash as a basal dose before transplanting.
The healthy seedlings were transplanted 35 days after sowing with a spacing of 15 cm between and 10 cm within the rows. The treatments were randomized within each replication. One month after transplanting, the crop was top-dressed with 25 kg of nitrogen per hectare in the form of urea. Regular plant protection measures were followed to check the incidence of pests and diseases as per recommendations (Anon, 1983).

2.3 Harvesting of Bulbs

The bulbs were harvested when foliage turned yellow and tops started falling. Harvesting of bulbs was staggered as and when bulbs matured.

2.4 Bulb Planting

Uniform healthy bulbs were selected per pot three bulbs were planted in pots. Bulbs were cut and fungicide (Blitox) paste was applied at top one-third portion of the bulb before planting. At the rate of 2g per litre of water Bulbs were drenched with captaf immediately. Control of pest and diseases have been done by taking regular plant protection measures. Each line consisted of 8 pots.

Visual observation, bagging, and staining techniques are used for identification of fertile and sterile lines. Staining of onion genotypes is said to be the most suitable technique for confirmation of sterile and fertile lines.

Preparation of the Alexander staining solution Add to a light-protected bottle: 10 ml 96% ethanol 10 mg Malachite green (1 ml of 1% solution in 96% ethanol) 50 ml distilled water 25 ml glycerol 5 gm phenol 5 gm chloral hydrate; 50 mg acid fuchsin (5 ml of 1% solution in water) 5 mg Orange G (0.5 ml of 1% solution in water) Glacial Acetic acid to the final concentration of 4% Keep in dark.

3. RESULTS

3.1 Identification of Male Sterile Lines in Genotypes

3.1.1 Visual observation method

Male sterile lines in each genotype are identified visually by the presence or absence of pollen by

dusting flowers into Petry plates and hand touching method. The screening was made manually by the hand-touching method. Plants were categorized as sterile or fertile based on the presence or absence of pollens. If the yellow-colored pollens attach to the hand, then that is identified as a fertile plant and if no pollens attach to hands, then they are considered sterile, usually sterile pollens are light green to black and fertile pollens are yellow male sterile lines Identified in five lines PWR-18, RW-18, AW-O-18, PWO-18, TW-18.

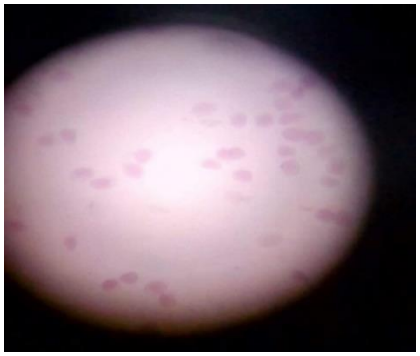
3.1.2 Bagging technique

Flowers were bagged before opening of the flowers. If the pollens are attached on the butter paper bag then the plant is fertile if no pollens are attached then the plant is sterile, one can clearly observe the presence of yellow pollens sticking on the butter paper bags. Selfing of pollens was done in all the flowers till the seed set. Butter paper bags were retained until the flowers formed seeds in the umbel and after the formation of the seed, the seeds were collected. Seed set is observed only in fertile pollens and there is no seed set in sterile flower.

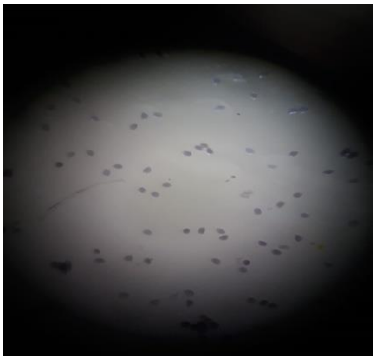
3.1.3 Staining technique

Pollens of individual flowers were collected in Petry plates and they were crushed by using a needle on a glass slide. One drop of 15 % sucrose media is added for growth and allowed for overnight incubation for pollen tube germination.

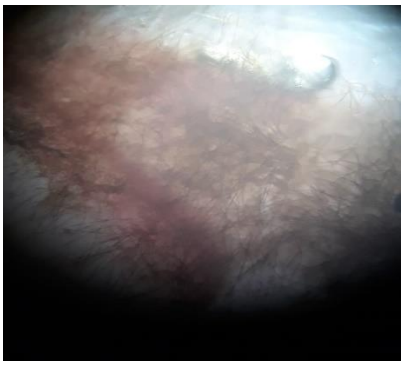
After adding of sucrose pollens were stained with Alexander stain pollens viability was accounted for under a microscope and sterile lines were identified. The fertile pollens had a stain on staining with the Alexander stain pink staining is observed and sterile pollens do not have any stain they are black after keeping the slide overnight pollen tube germination was observed in fertile pollens followed by no pollen germination in sterile pollens. (Plate 1 to Plate 5).



PWO-18 –Fertile pollen

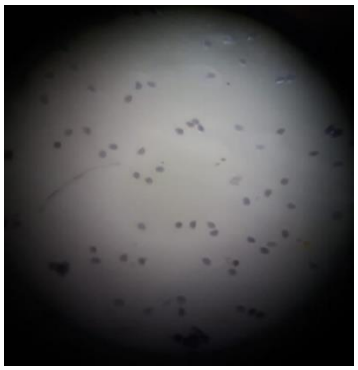


PWO-18-Sterile pollen



PWO-18 Fertile pollen

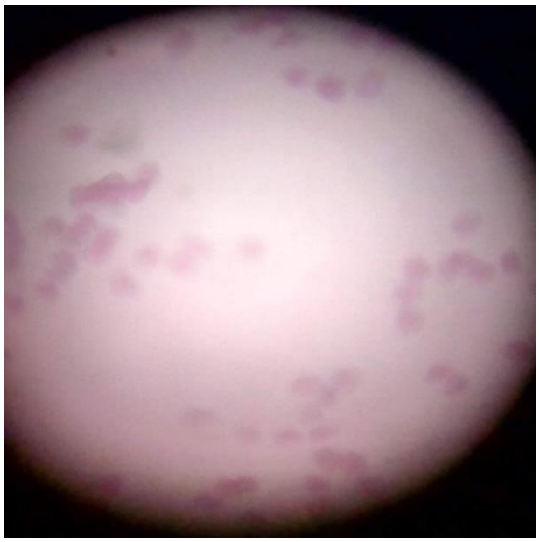
Pollen tube formation in fertile with Staining



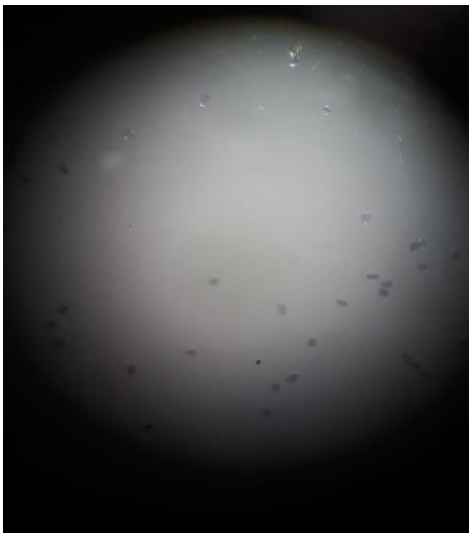
PWO-18 Sterile pollen

No pollen tube formation with Staining

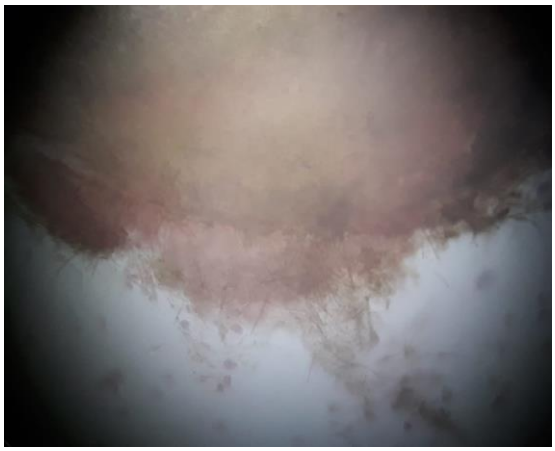
Plate 1. Pollen tube formation (PWO-18)



TW-18 -Fertile pollen

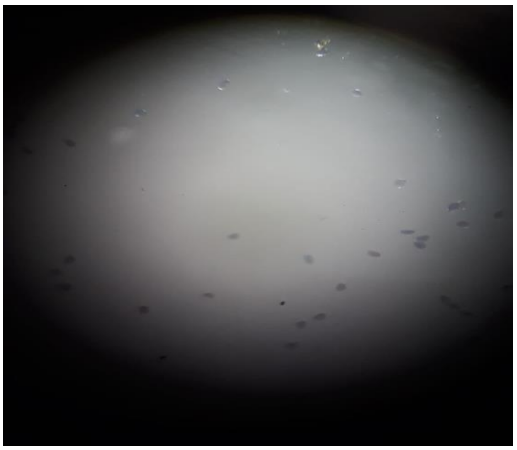


TW-18 -Sterile pollen



TW-18 Fertile pollen

Pollen tube formation with staining



TW-18 Sterile pollen

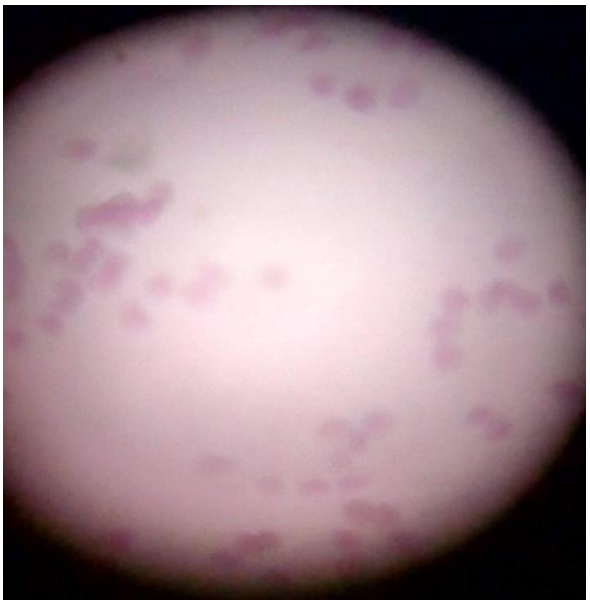
No pollen tube formation with staining

Plate 2. Pollen tube formation (TW-18)

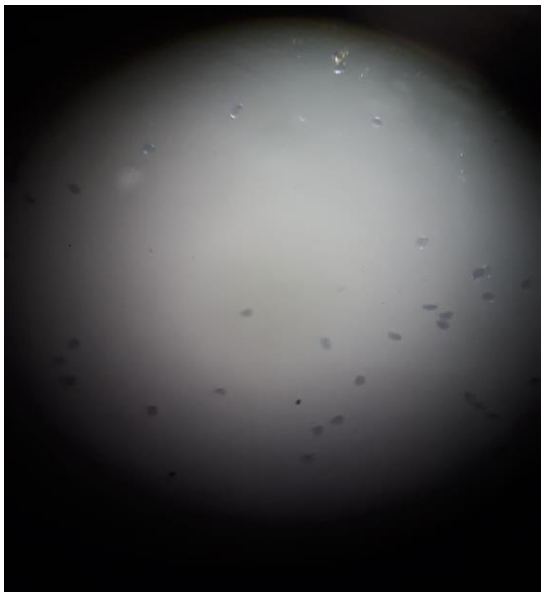
Table 1. Pollen tube growh response of genotypes for staining

Sl no	Genotypes	Response to staining
1	PWO-18 MS	–
2	PWO-18 MF	+
3	TW-18 MS	–
4	TW-18 MF	+
5	PWR-18 MS	–
6	PWR-18 MF	+
7	RW-18 MS	–
8	RW-18 MF	+
9	AW-O-18 MS	–
10	AW-O-18 MF	+

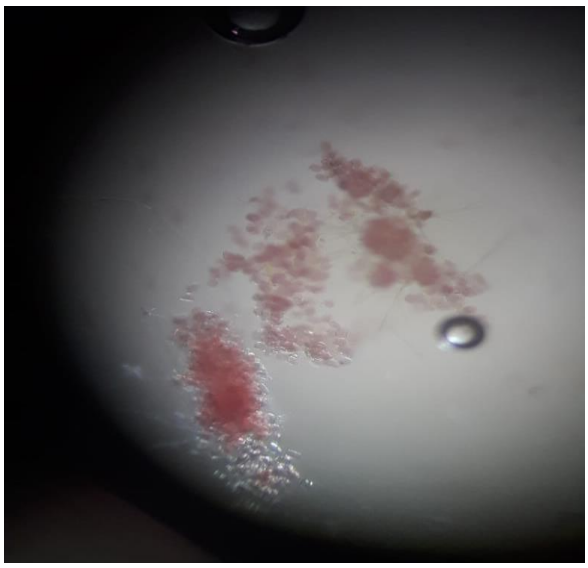
MS- Male sterile; MF-Male fertile; + Stained; - No stain



PWR-18- Fetile pollen

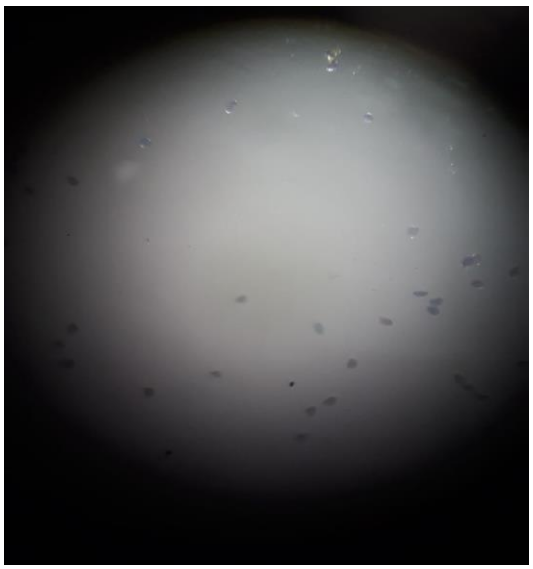


PWR -18 Sterile pollen



PWR-18 Fertile pollen

Pollen tube formation with staining



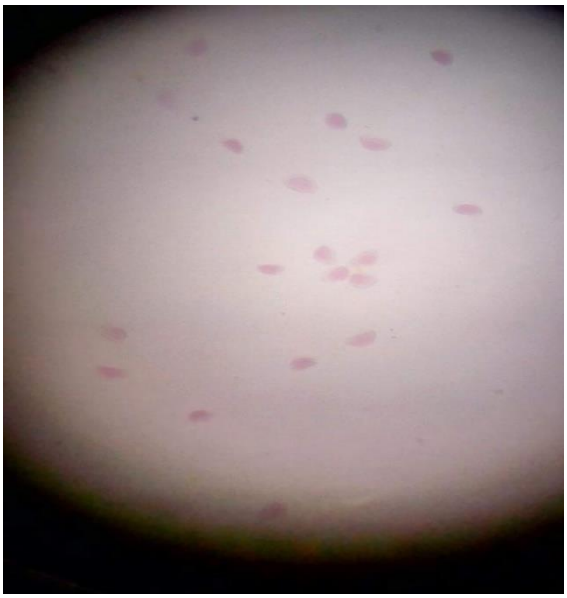
PWR-18 Sterile pollen

No pollen tube formation with staining

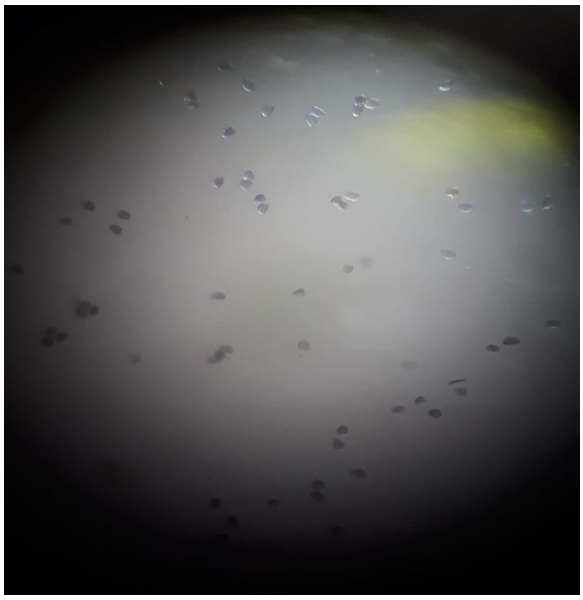
Plate 3. Pollen tube formation (PWR-18)

4.5 Floral characterization of male sterile lines

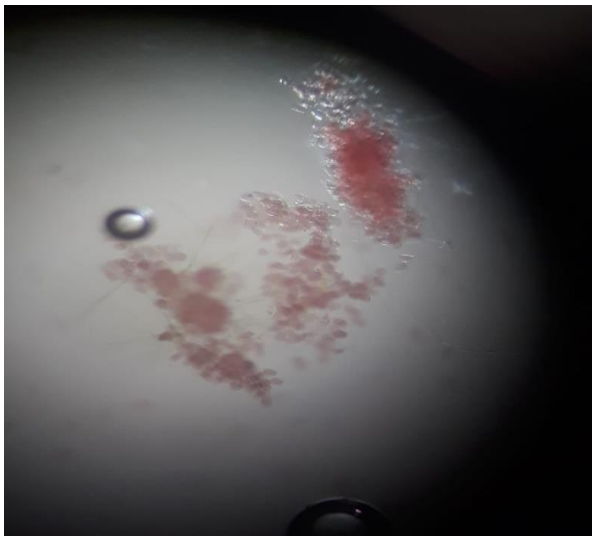
The sequence of development of individual flowers were studied bud formation, Flower initiation, opening of flower, seed set and withering of flowers are noted and given in plate (8 and 9) Flowering takes 64-67 days to flower from the date of bulb sowing, remain flowering for next 40-45 days and 88-91 days to produce maximum number of open flowers. Anthesis occurs at 6-7 am and anther dehiscence continues from 7 am to 5 pm. Each anther produces an average of 1609 pollen grains.



RW-18- Fertile pollen

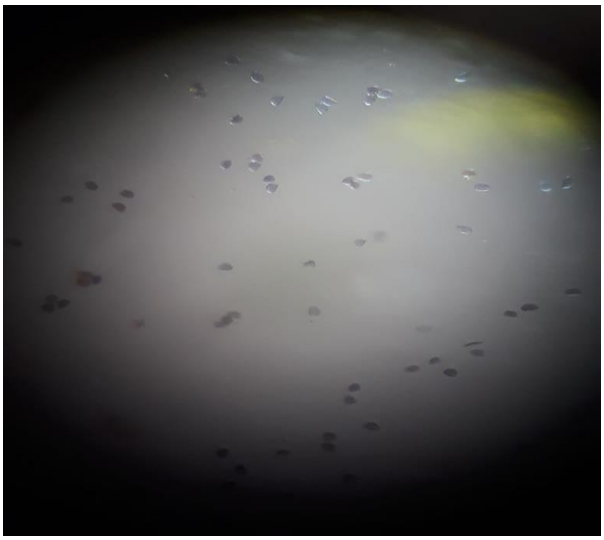


RW-18- Sterile pollen



RW-18 Fetile Pollen

Pollen tube formation with staining



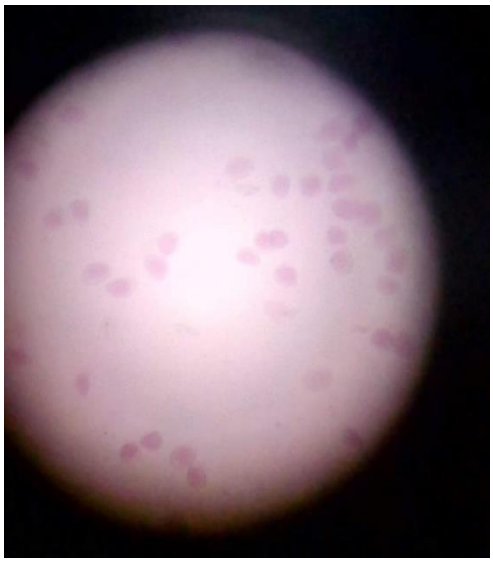
RW-18 Sterile pollen

No pollen tube formation with staining

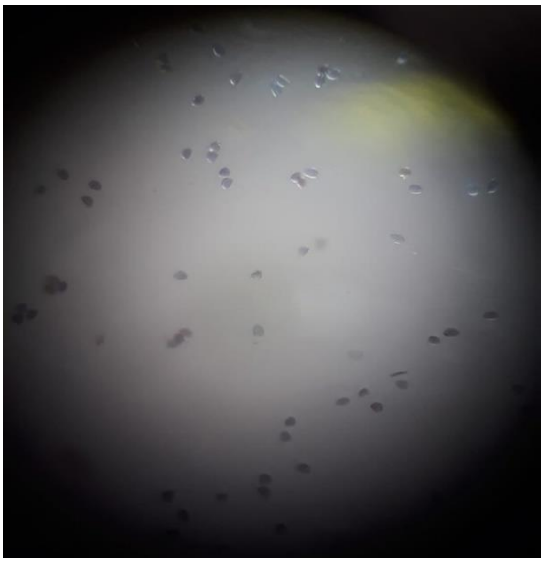
Plate 4. Pollen tube formation (RW-18)

At anthesis, two classes of flowers were identified. These were flowers with fully extended stigmas greater than 4 mm long and open petals and flowers in which the stigmas did not extend more than 2 mm and petals that did not fully open were observed in early morning time by opening of butter paper bags in the umbel.

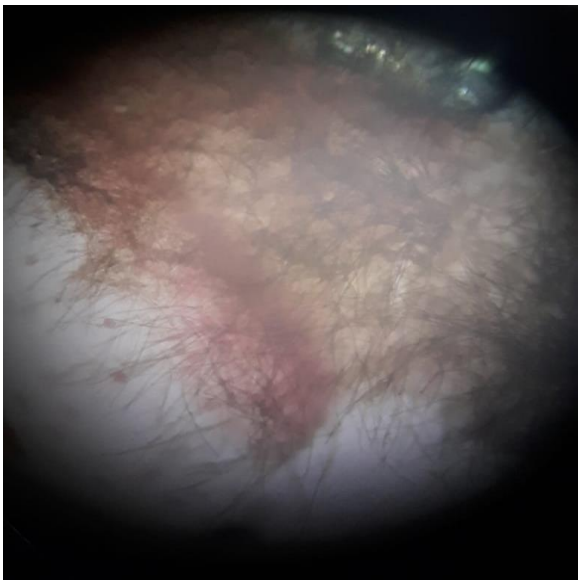
The morphological characteristics of the developing flowers differed markedly between aborted and normally developing flowers, the first visible sign occurred prior to petal opening when elongation of the style through the petal gap at the tip of the bud was evident in normally developing flowers but negligible in aborting flowers. Normal and aborting flowers observed at this stage displayed differences in the surface morphology of the stigma, style and ovary surface. Aborting flowers displayed areas of desiccated tissue in all floral parts while normally developing flowers consisted of fully turgid structures



AW-O-18-Fertile pollen

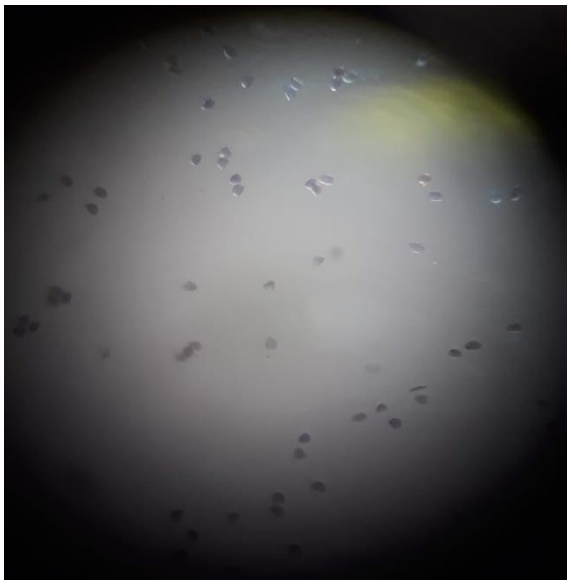


AW-O-18-Sterile pollen



AW-O-18-Fertile pollen

Pollen tube growth with staining



AW-O-18-Sterile pollen

No pollen tube formation with staining

Plate 5. Pollen tube formation (AW-O--18)

Male sterile and fertile lines varied in their stigma,style characteristics and development pattern. The styles of male sterile lines were longer during time of opening of flower and the stigmatic knob became receptive relatively sooner. Individual lines differed for time of flowering.

Six stages of flower development were defined, covering of umbels to the period from flower opening to withering of the style. Regular counts were made for the total number of flowers at each stage during the flowering. The pattern of flowering was similar in all cases, although the number of flowers per head varied from 126 to 681. At first, the number of flowers opening was small, but rapidly increased, giving a peak, which lasted for a mean of 4-10 days. During the middle of the peak flowering period, the maximum number of flowers opened at one time was often more than 50% of the total flowers on the head.

Table 2. Meterological data during the crop growth period (monthly)

Monthly average	Temperature,0c		Relative Humidity,%		Evaporation,mm	Wind speed, km/h	Rainfall, mm
	Max	min	8:30 AM	1:30 PM			
July	28.16	20.45	82.06	66.65	4.26	7.69	88.55
August	28.79	19.50	83.06	60.81	4.10	2.52	87.75
September	29.59	19.39	83.07	58.13	4.41	1.28	116.90
October	29.58	16.75	77.13	50.26	4.26	1.53	53.40
November	29.11	15.33	83.17	50.83	4.15	0.84	17.30
December	28.74	15.02	83.10	49.30	3.88	1.41	2.50
January	29.05	9.50	85.23	33.35	4.57	3.71	1.00
February	31.56	14.86	72.07	36.32	5.85	4.89	11.40
March	34.62	17.90	64.97	29.26	7.50	4.42	0.00
April	35.51	20.43	69.33	32.85	7.37	4.25	28.70

PWO-18 Sterile umbel

PWO-18 Fertile umbel

TW-18 Sterile umbel

TW-18 Fertile umbel

Plate 6. Sterile and fertile umbel of TW-18 and PWO-18

PWR-18 –Sterile flower umbel

PWR-18 –Fertile umbel

RW-18 –Sterile umbel

RW-18 –Fertile umbel

Plate 7. Sterile and Fertile umbel of TW-18 and RW-18

Flower opening started slowly, then rapidly built up to peak, followed by a rapid decline, which usually tailed off at the end of the flowering period. Early in the season, an umbel of PWR-18 showed two peaks of flowering instead of a single peak followed by Telagi white and RW-18. Between the two peaks of flowering was associated with a drop of flowering at the end.

During different stages of flower development, 6 Stages of flower development were distinguished. The stages are defined in during stage 2 there was rapid elongation of the style, but no sign of a stigmatic knob. The inner whorl of stamens disappeared before the outer, and there was usually a pause of up to 24 hours between these two events. During stage 3 the style was about the same length as the stamens, but in stage 4 the stigma was above the general level of the anthers. The stigmatic knob became apparent during stage 4, and the surface of the stigma appeared to glisten, indicating the secretion of stigmatic exudate. During this stage the style remained turgid and the stigma reached its greatest size, but by the end of this stage, the filaments were almost completely withered. All the floral parts, except for the ovary were withered by the end of stage 6. During this stage, growth of ovaries with fertilized ovules continued,

but unfertilized ovaries began to dry up in sterile plants.

4. DISCUSSION

In recent days cultivation of F_1 hybrids has gained fore most importance due to their productivity and economic returns. Male sterile lines are essential to develop F1 hybrids. Identification of male sterile parent which help to facilitates in early development of F1 hybrids. White onion was selected for the present study considering its importance for processing industry, for local and export markets and there is immense scope available for exploitation of heterosis. Till date, several MS lines have been identified and reported but male sterile line in white onion background is not available. The results obtained from these investigations are discussed below.

Onion is strongly protandrous, self-pollination is largely absent which depends upon insects for cross pollination inflorescence is present at the plant top which is hollow from the inside. The height may differ from 0.9 m to 1.2m depending on the genotype and other factors. Flowers in umbel are covered by a membranous 2-3 white coloured sheath called spathe. The sheath splits because growing flowers create pressure inside the umbel. There may be 50 - 2000 florets in an Inflorescence depending on the genotype, planting time, size and conditions of storage of mother bulb. The flowers are white in colour. The segments in the perianth are six in two whorls spreading, reflexed, free and ovate. There are also six stamens in two whorls. Anthers are bilocular and the ovary is superior. Anthers may be green or yellow in colour depending upon genotype. The colour also changes with the age of flower. Some florets have two ovaries with seven stamens. Anthesis occurs in early morning (6-7 h) dehiscence of anther takes place between 7.00 and 17.00 h and on next day also with the peak between 9.30 and 17.00 h. on the day of anthesis pollen fertility is more as well as receptivity of stigma is on the day of anthesis.

Male sterile lines in each background are identified visually by presence or absence of pollen by dusting of flowers into petry plates. Screening was made manually by hand touching method. Plants were categorized as sterile or fertile based on presence or absence of pollens. Pollens of selected lines were collected and they were crushed by using a needle on a glass slide one drop of 15 % sucrose medium was added for growth and after overnight incubation for pollen tube germination they were stained with Alexander stain and pollens viability was accounted under a microscope and sterile lines are identified.

The fertile pollens had a stain on staining with the Alexander stain and sterile pollens do not have any stain after keeping the slides overnight pollen tube germination was observed in fertile pollens followed by no pollen germination in sterile pollen. Then the selected lines were tagged for further studies of the sterile and fertile plants.

Characterization of male sterile lines and fertile lines we observed the differences among male sterile lines and fertile lines in the plants the flowering behavior, time of anthesis, pollen bearing of the umbels and the colour of pollens in the umbels help to characterize the plants based on the floral behavior of the plants.

Onion flower bud

Onion flower opening

Full opened flower

Seed set in flower

Florogenesis in onion flower from bud to seed

Plate 8. Florogenesis in onion flower

Flower bud in umbel

Flowers opening in umbel

Full opened flower in umbel

Seed formation in umbel

Plate 9. Sequence of flowering to seed formation in onion umbels

Floral studies in onion have previously described differences in reproductive structure between male-sterile and male fertile lines; differences in style length between male-sterile and male fertile lines have been documented and suggested as a possible reason for poor seed set (Robert, 1954). The receptive area of the stigmatic knob has also been shown to be comparatively less in case of male-sterile lines [29]. The receptivity period of the onion flower has also been considered as a factor affecting seed set and the style length might also be related to the receptivity period (Robert, 1954). Similar

characters were observed in selected genotypes.

The morphological characteristics of the developing flowers differed markedly between aborted and normally developing flowers, the first visible sign occurred prior to petal opening when elongation of the style through the petal gap at the tip of the bud was evident in normally developing flowers but negligible in aborting flowers. Normal and aborting flowers observed at this stage displayed differences in the surface morphology of the stigma, style and ovary surface. Aborting flowers displayed areas of desiccated tissue in all floral parts while normally developing flowers consisted of fully turgid structures

The number of flowers in an umbel varied greatly but was commonly in the range 100-600. The flowers were tightly packed, and about a week or 10 days after the start of flowering, the opening of new flower buds tended to displace adjacent flowers which had opened previously. Flowers which opened later, developed longer pedicels and consequently the earlier flowers became buried, once they reached stage 6, under a layer of more recent flowers [10,29,3-9,18-23,11-17,24-28,30-32].

The general pattern of flowering found by us was very similar to that of the total flowering period lasted 29 days, and in the 15-day period during the middle of flowering 85% of the flowers were open.

Although our records were made on single flowering heads, it is quite common for a single onion plant to have more than one umbel. The number of umbels per plant can be related to bulb size and to cultivar. In plants with more than one umbel, the umbels often start flowering within a few days of each other, but occasionally a week or more may elapse between the start of flowering of the first and last umbel [33-37].

5. CONCLUSIONS

The staining method was found to be a more reliable method for the identification of male sterile lines as compared to other methods under study.

Staining was observed most prominent in PWO-18 followed by AWO-18

ACKNOWLEDGEMENT

The authors are thankful ICAR-IIHR Division of vegetable crops and to the Department of Vegetable Science, Indira Gandhi Krishi Vishwavidhyalaya, Raipur (C.G.).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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