

Identification of male sterile lines and floral characterisation of white onion (*Allium cepa*)

Abstract:

The present was carried out at the Division of Vegetable crops, IIHR- Bengaluru. The experiment was carried out with Randomized block design with 10 treatments and 3 replications namely PW0-18, TW-18, PWR-18, RW-18, 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

Comment [mm1]: On abstract: please add introduction, problems, aims, methods, results, and novelty

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Introduction:

Onion is the commercial vegetable of India with huge foreign exchange earnings (Over 1443.09 crores). Genetic improvement of onion will affect the national economy in a positive way. Jones and Mann (1963) have suggested Pakistan, Iran, and the mountainous areas of north India as its origin. India has an area of 12.30 Lakh ha in the world with production of 215.64 lakh tons and productivity of 16.16t/ha (NHB, 2016-17). leading producer of onion is Maharashtra followed by Karnataka and Madhya Pradesh. India stands third in export. (NHB, 2018).

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 pistil and 6 perianth segments. The pistil has 3 locules each of which has 2 ovules. Anthesis occurs early in the morning at 6-7 am and anther dehiscence continues after anthesis at 7 am to 5 pm. Yellow anthers have a greater number of pollens we can observe followed by a smaller number of pollens in the green anthers comparatively.

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Material and Methods:

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 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 complete germination of seeds. The seedlings were ready for transplanting after forty-five days.

Preparation of main field

The experimental field is made in to fine tilth by repeated ploughing, clodding and removing weeds from the field. Farmyard manure was incorporated into the soil at the rate of 25 tonnes per hectare and land was perfectly levelled. The uniform plots of size 2m x 1.0m were prepared. Fertilizers at the rate of 75 kg of nitrogen, 35 kg of P₂O₅ and 50 kg of K₂O 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 the spacing of 15 cm between and 10 cm within the rows. The treatments were randomised 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).

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.

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 technique are used for identification of fertile and sterile lines. Staining of onion genotypes is said to be 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.

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Result and Discussion:

Identification of male sterile lines in genotypes

Visual observation method

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

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 forms seed in the umbel and after the formation of seed the seeds are collected. Seed set is observed only in fertile pollens and there is no seed set in sterile flower.

Staining technique

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

After adding of sucrose pollens were stained with Alexander stain and pollens viability was accounted under microscope and sterile lines are identified. The fertile pollens had a stain on staining with the Alexander stain pink staining is observed and sterile pollens does not have any stain they are black in colour 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).

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Conclusions

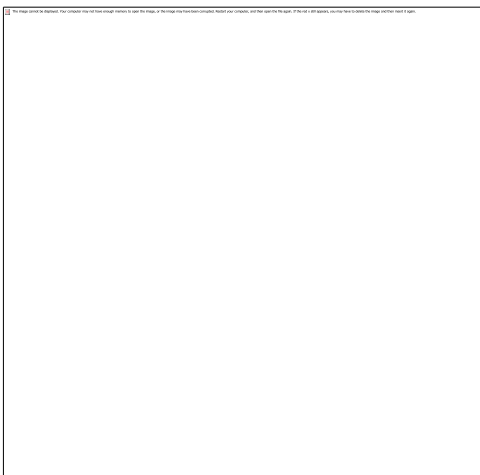
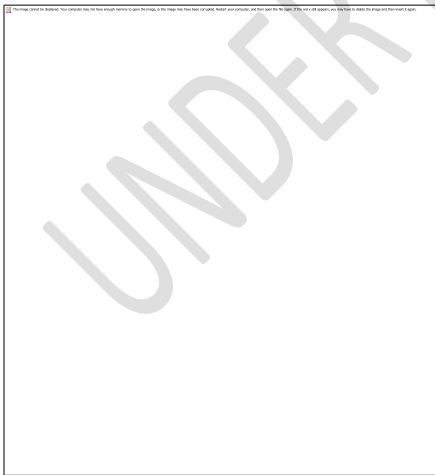
1. Staining method was found to be more reliable method for identification of male sterile lines as compared to other methods under study.

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

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Acknowledgment:

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



UNDER PEER REVIEW

PWO-18 –Fertile pollen

PWO-18-Sterile pollen



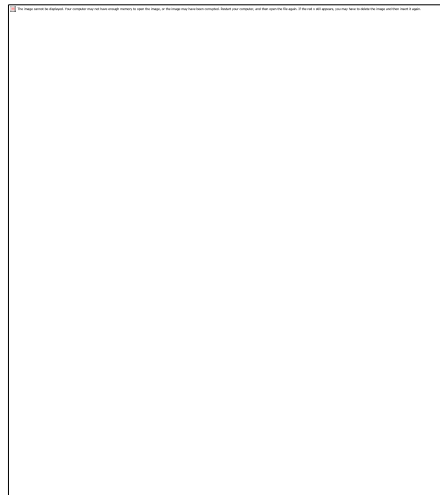
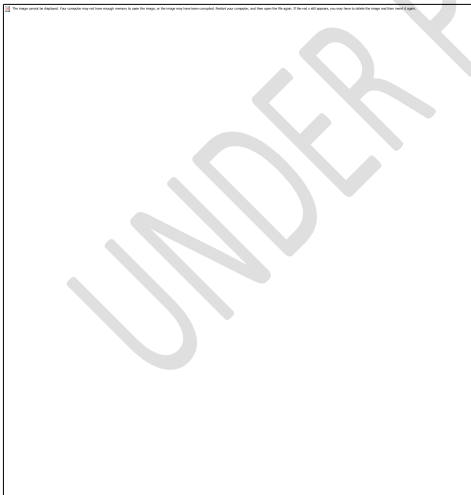
PWO-18 Fertile pollen

PWO-18 Sterile pollen

Pollen tube formation in fertile with Staining

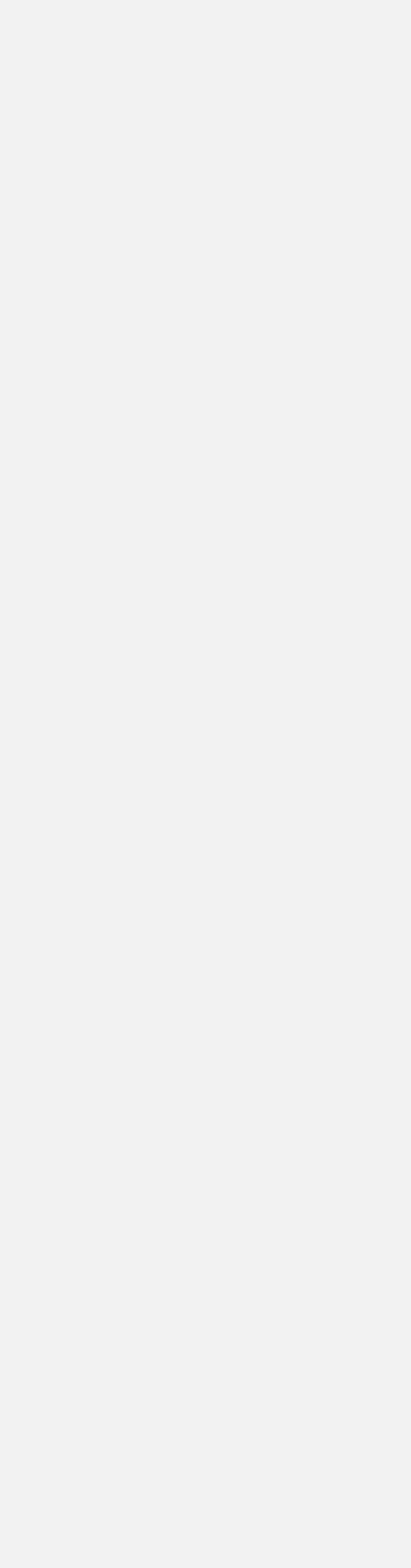
No pollen tube formation with Staining

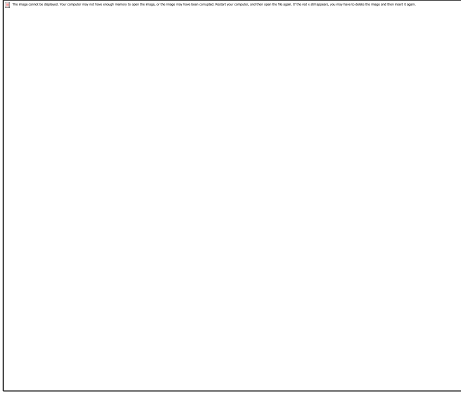
Plate 1.Pollen tube formation (PWO-18)



TW-18 -Fertile pollen

TW-18 -Sterile pollen





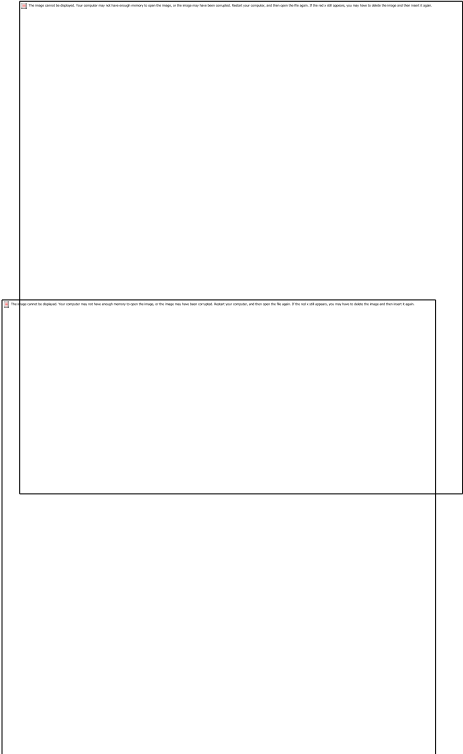
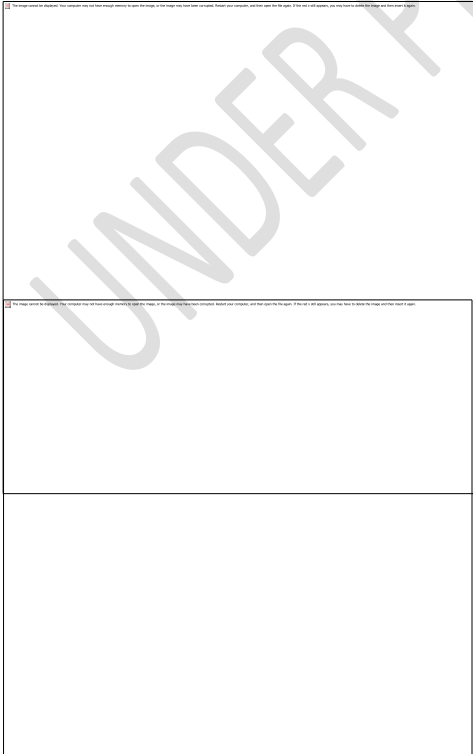
TW-18 Fertile pollen

TW-18 Sterile pollen

Pollen tube formation with staining

No pollen tube formation with staining

Plate 2.Pollen tube formation (TW-18)



PWR-18- Fetile pollen

PWR-18 Fertile pollen

Pollen tube formation with staining

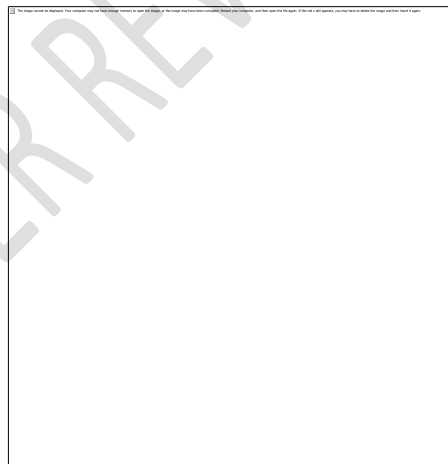
Plate 3.Pollen tube formation (PWR-18)



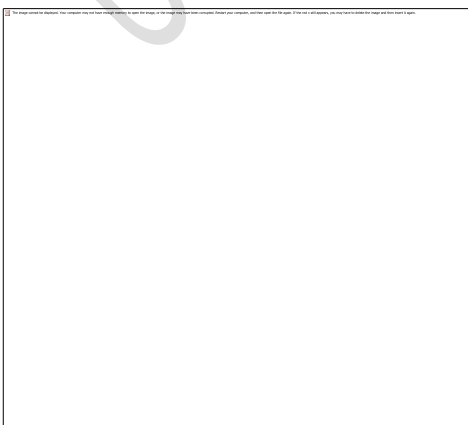
PWR -18 Sterile pollen

PWR-18 Sterile pollen

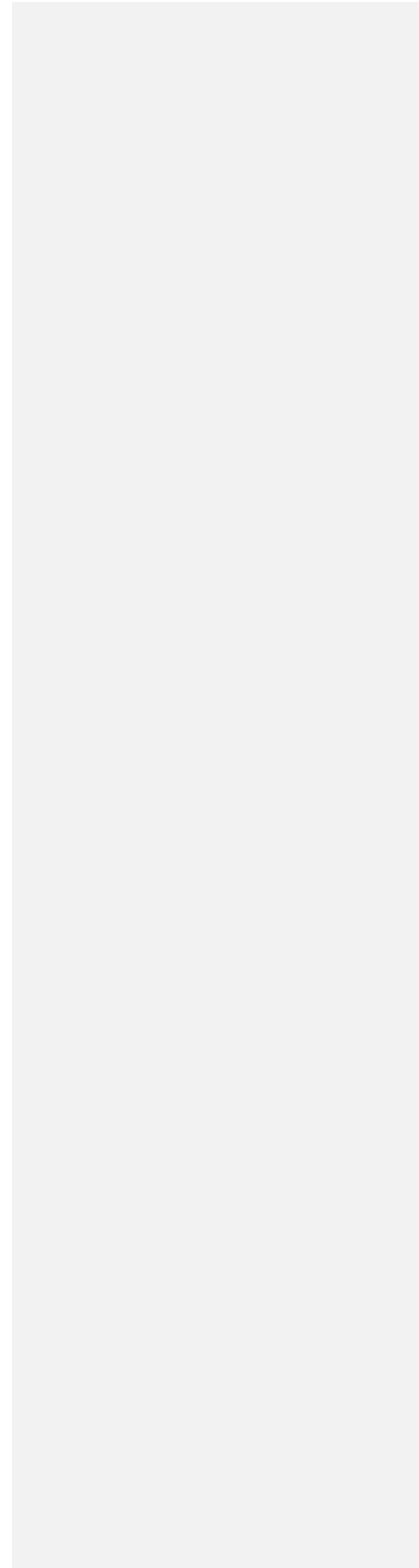
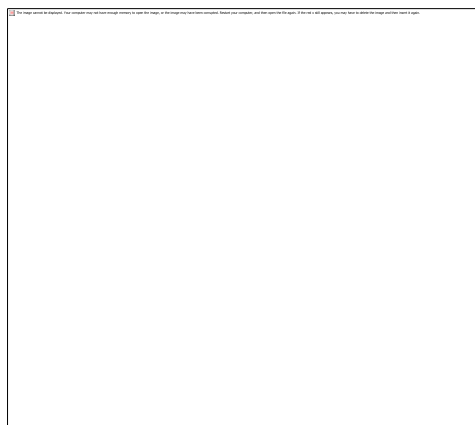
No pollen tube formation with staining



RW-18- Fertile pollen



RW-18- Sterile pollen



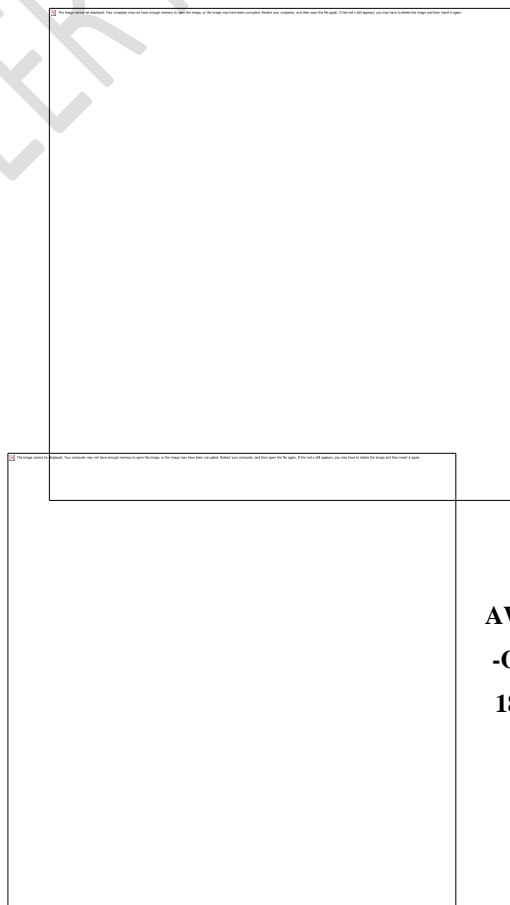
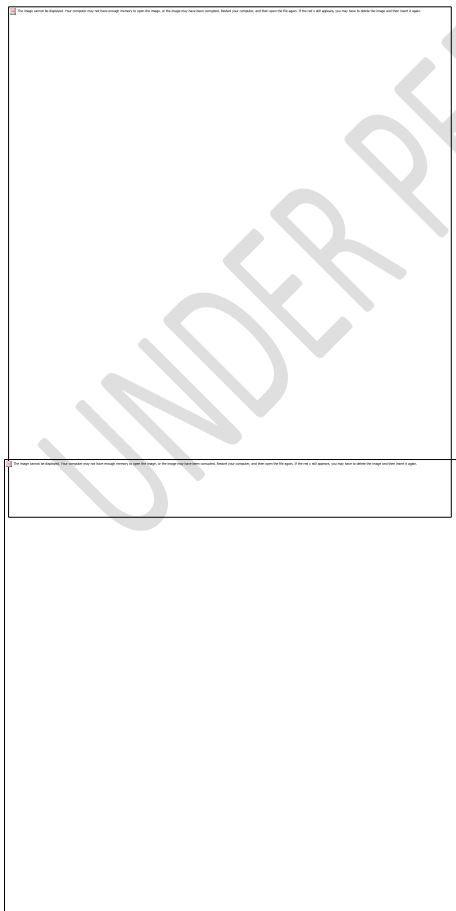
RW-18 Fetile Pollen

Pollen tube formation with staining

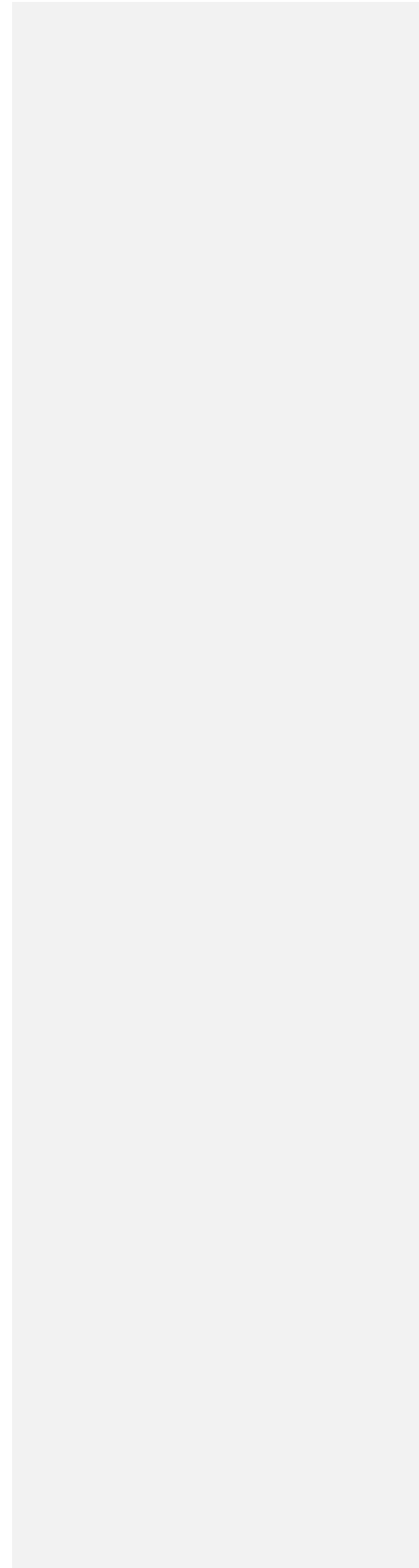
Plate 4.Pollen tube formation (RW-18)

RW-18 Sterile pollen

No pollen tube formation with staining



**AW
-O-
18-**



Fertile pollen

AW-O-18-Sterile pollen

AW-O--18 Fertile

AW-O-18- Sterile

Pollen tube growth with staining

No pollen tube formation with staining

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

Table 1. Pollen tube growth 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

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

monthly average	Temperature, 0c		Relative Humidity,%		Evaporation,m m	Wind speed,km/ h	Rainfall,m m
	Max	min	8:30	1:30			
			AM	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

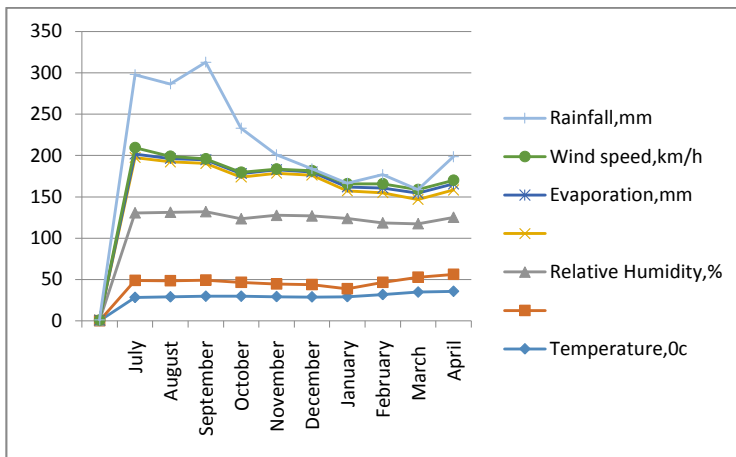


Fig 1. Graphical presentation showing Meteorological data during the crop growth

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 in 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.

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 extended 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

Male sterile and fertile lines varied in their stigma and 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.

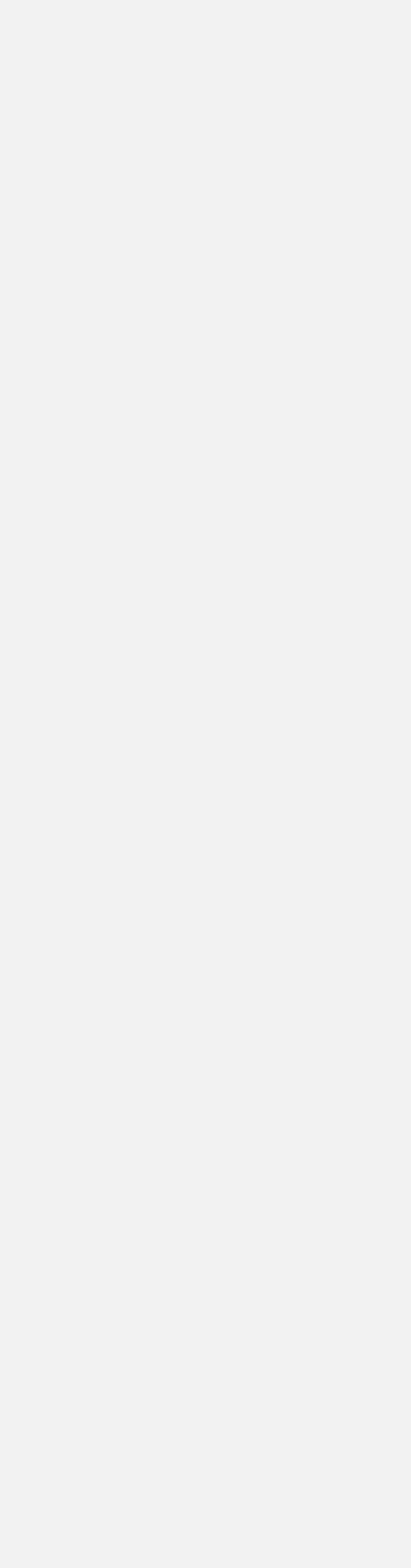
Six stages of flower development were defined, covering of umbels to the period from flower opening to withering of the style. Regular counts were made for the total number of flowers at each stage during the flowering. The pattern of flowering was similar in all cases, although the number of flowers 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 opens at one time was often more than 50% of the total flowers on the head.

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 the 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 dehisced before the outer, and there was usually a pause of up to 24 h 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 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 plant.

UNDER PEER REVIEW



PWO-18 Sterile umbel

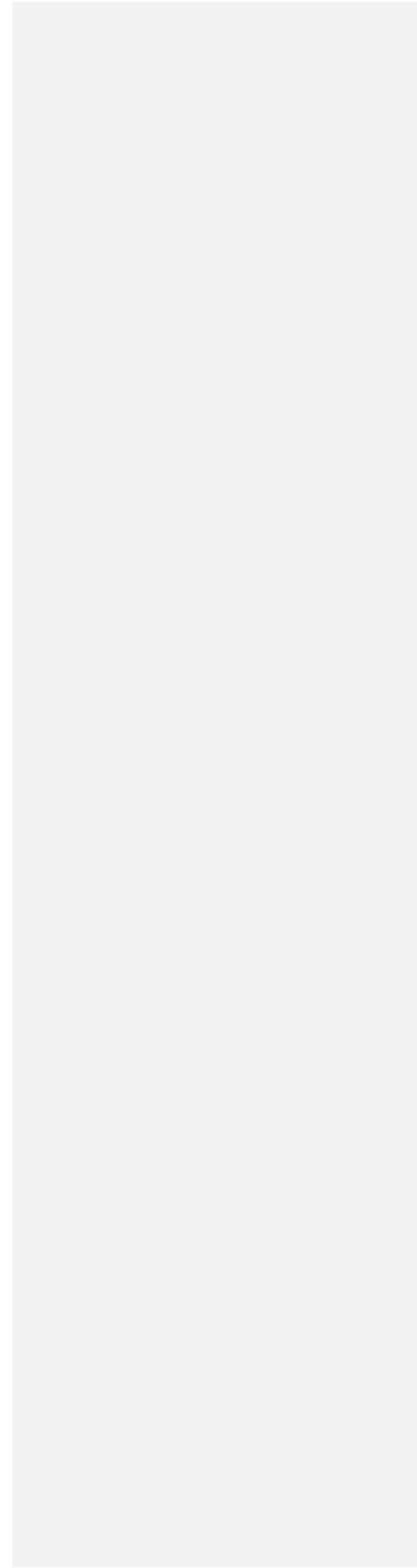
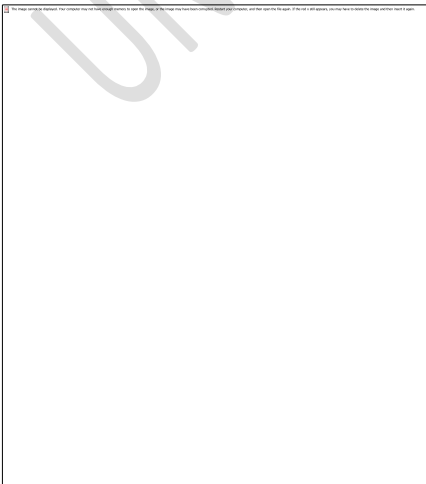
PWO-18 Fertile umbel



TW-18 Sterile umbel

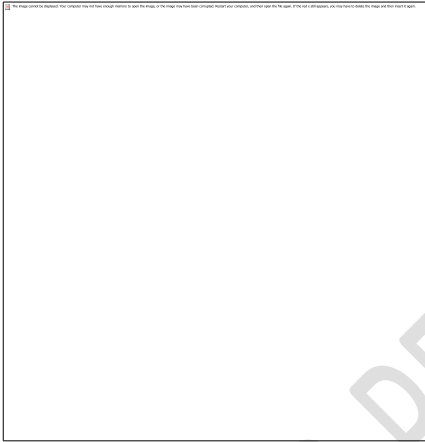
TW-18 Fertile umbel

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



PWR-18 –Sterile flower umbel

PWR-18 –Fertile umbel

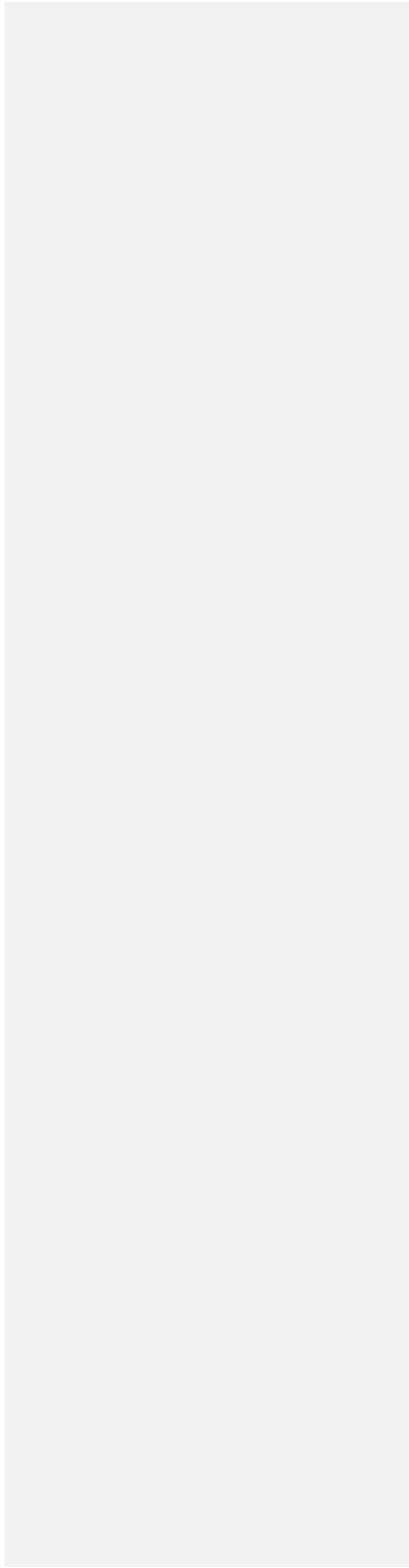


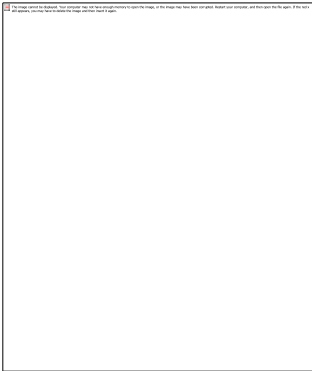
RW-18 –Sterile umbel

RW-18 –Fertile umbel

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

UNDER PEER REVIEW





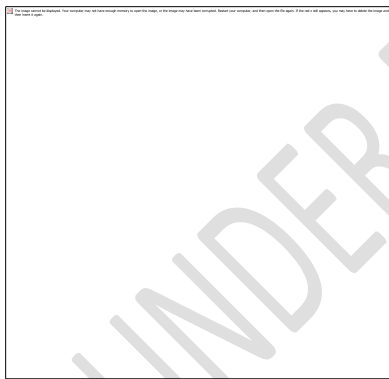
Onion flower bud



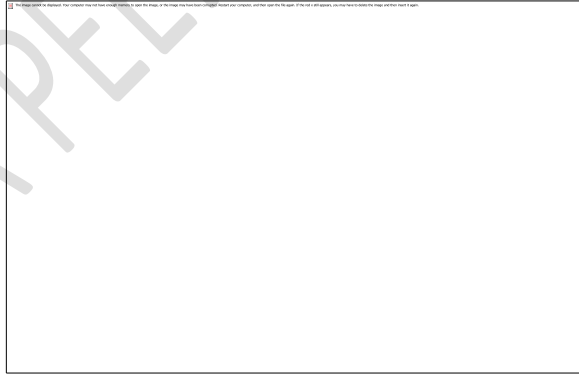
Onion flower opening



Full opened flower



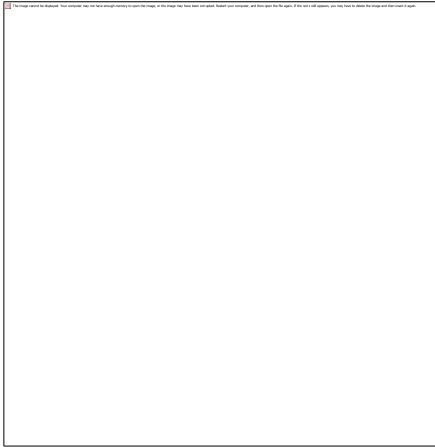
Seed set in flower



Florogenesis in onion flower from bud to seed

Plate 8. Florogenesis in onion flower

UNDER PEER REVIEW



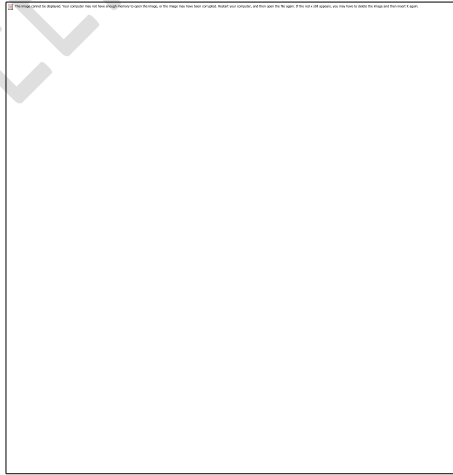
Flower bud in umbel



Flowers opening in umbel



Full opened flower in umbel



Seed formation in umbel

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

Discussion

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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 F_1 hybrids. Identification of male sterile parent which help us to facilitates in early development of F_1 hybrids. White onion was selected for the present study considering its importance for processing industry, for local and export market 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 line in each background are identified visually by presence or absence of pollen by dusting of flowers in to 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 are crushed by using a needle on a glass slide one drop of 15 % sucrose medium is added for growth and after overnight incubation for pollen tube germination they are stained with Alexander stain and pollens viability was accounted under microscope and sterile lines are identified.

The fertile pollens had a stain on staining with the alexander stain and sterile pollens does 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 helps us to characterize the plants based on the floral behavior of the plants.

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 (Ali et al., 1984). 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

The general pattern of flowering found by us was very similar to that 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.

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