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Pollination and fruit setting mechanism in spine gourd (*Momordica dioica* Roxb)

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Abstract

Information on fruit setting in spine gourd (*Momordica dioica* Roxb) has received little attention, and the precise nature of its breeding system has never been described. A study was undertaken on the pollination mechanism, fruit setting, for genetic improvement of spine gourd. The first flower produced by the male plant reached full bloom 35 days after emergence from the soil; those produced by female plant required an average of 45 days. Number of nodes producing the first flower varied from plant to plant and subsequent flowers also did not develop in any definite sequence. The time required by male and female flower buds to reach anthesis was 12 and 18 days, respectively. Opening of either type of flower occurred between 4 and 7 AM, and dehiscence of anthers began at time of opening of female flower. The stigma became receptive 14 hours before anthesis. This study may provide information for development of hybrids and accelerate breeding procedure of spine gourd.

Keywords: Pollination, fruit setting, spine gourd, *Momordica dioica*

Introduction

Spine gourd (*Momordica dioica* Roxb.) belongs to family cucurbitaceae. It is distributed in India, Bangladesh, China, Malaysia, Nepal, Myanmar, Pakistan and Srilanka (Rakh and Chaudhari, 2010) [12]. This is a highly demandable seasonal vegetable and medicinal plant in Asian countries. This species is indigenous to India; because of its higher medicinal importance it is demandable vegetation throughout the world (De Wilde & Duyfjes, 2002; Joseph, 2005; Joseph & Antony, 2008) [2, 6, 7]. The medicinal importance of spine gourd is sex-specific and only female plants have medicinal values (Sastri *et al.*, 1962) [13]. The fruit giving plants have its own value in preparation of medicines and leaves of female spine gourd are used as an aphrodisiac, to eliminate the parasites present in the human intestine, cure fever and respiratory disorders, Kumar & Prajapati (2003) [8]. The root and tubers are used for the treatment of headaches, kidney stones and jaundice. Kumar & Prajapati (2003) [8] Medicinal value of this plant was also these researchers reported that fruits are useful in the treatment of asthma, leprosy, fever, tumors, urinary discharges, excessive salivation, and heart disease, Jain & Singhai (2010) [4]. There is a lot of evidence showing that eating plenty of fruits and vegetables is good for our health. It is necessary to investigate those plants which have been used in traditional medicine that have to improve their quality of healthcare. Over the past 30 years, researchers have developed a solid base of science to back up what generations of mothers preached. Vegetables provides a diverse range of tastes, aromas, texture, colours and nutritional attributes, it increase the variety in the food (Diane *et al.*, 2010) [3]. Vegetables are good source of vitamins, minerals and dietary fiber. Vegetables play a significant role in human nutrition, especially as sources of vitamins C (Ascorbic acid), Thiamine (B1), Niacin (B3), and Pyridoxine (B6), Folacin (also known as folic acid or folate) (B9), minerals and dietary fiber (Craig and Beck, 1999 Quebedeaux and Eisa, 1990; Wargovich, 2000) [1, 11, 17]. Vegetables in the daily diet have been strongly associated with reduced risk for some forms of cancer, heart disease, stroke, and other chronic diseases (Southon, 2000; Tomas-Barberan and Espin, 2001) [15, 16].

As a dioecious perennial crop, fruit setting requires transfer of pollen from the male flowers of the male plant to the female flowers of the female plant and therefore proximity between male and female plants is desirable. An observation on ex situ cultivation in Kerala indicated less than 5% fruit set under natural conditions and nearly 100% with hand pollination (John *et al.*, 2007) [5]. Reported only 22% fruit set under normal cultivation, which indicates pollinator specificity. In order to apply the artificial pollination method effectively, the floral biology of spine gourd should be known.

Improvement of this crop has been not attempted, perhaps because of its dioecious nature and vegetative mode of propagation. Kankoda has a number of problems relating to its yield and fruit quality among the problems low yield, small sized fruit, low bearing lack of synchronized male and female plants, regular some hand pollination, and presence of large number of hard seeds in the fruit are considered to be important. Spine gourd reported that homosexual crossing in Kankoda set seeds, which may serve as a source of variation for commencing true breeding programme.

Material and Methods

The investigation was conducted at research cum institutional farm, Rajmohini Devi College of Agriculture & Research Station, Ambikapur, Chhattisgarh during *kharif* 2016-17 and 2017-18. This is located at latitude of 20° 8' N, longitude of 83° 15' E and altitude of 613.07 m MSL (Mean Sea Level). The place of investigation is a sub-humid region. It receives 1130 to 1250 mm rainfall annually; out of which above 88% is received during the rainy season (June to September).

Result and Discussion

Pollination mechanism and fruit setting

The floral behavior studies on spine gourd (*Momordica dioica* Roxb.) were carried out in *kharif* season (2017-18). The single flower bud appeared in every node on both male and female plant. The male flowers began to appear from the second week of August and differentiation continued till the first week of October, while, female flowers opened from the third week of August and continued till the end of September. The pedicle of male flower buds elongated and so they were pushed above the other buds of a vine. Soon these buds swelled and assumed a cup shape. The flower was taken to have opened as soon as the perianth leaves separated from each other. The dehiscence started with the appearance of a slit on the anther lobe which resulted into liberation of the creamy mass of pollen grains on the surface of anther.

Anthesis

Anthesis is the period during which a flower is fully open and functional. The anthers dehisced at the opening of flowers and release of pollen grains continued for several hours. Prior to dehiscence, anthers became shiny in appearance. Commencement of dehiscence was detected by the appearance of longitudinal slits on the middle of the anther lobe, which widened further, and pollen was seen busting out in creamy yellow, sticky masses deposited on anther walls.

Anthesis time

There were variations in anthesis time for different genotypes, the male plant anthesis started as early morning around 4:00 AM while female plants flowered during 6:00 to 6:20 AM and no anthesis was recorded beyond 6:20 AM in any plant. The period from 5:00 to 6:00 AM could be regarded as peak anthesis time of spine gourd.

The anthesis time of the day in different spine gourd genotype has been summarized in table 3 and 4. The anthesis of the pistillate flowers was occurred earlier than the staminate flowers. The data of anthesis was recorded at 5 intervals: 04:15 to 04:45, 04:45 to 05:15, 05:15 to 05:45, 05:45 to 06:15 and 06:15 to 06:45 AM.

Anthesis in most of the genotypes was started during the period of 04:45 to 05:15. During the period of 04:15 to 04:45 the genotype AMBIKA 13-6 was showed maximum 40% anthesis followed by AJSG-1 with 37% and RMDSG-3 with

36% anthesis and during the period of 04:45 to 05:15 in the genotype CHHATTISGARH KANKODA-2 recorded maximum 50% of anthesis followed by INDIRA KANKODA-1 with 48% of anthesis and AMBIKA 13-6 with 45% anthesis and during the period of 05:15 to 05:45 in the genotype NMD-5 recorded maximum 30% of anthesis followed by NDM-2 and AMBIKA 13-5 with 20% of anthesis and during the period of 05:45 to 06:15 in the genotypes AJSG-1, RMDSG-4, AJSG-2 and CHHATTISGARH KANKODA-2 recorded maximum 10% of anthesis followed by NDM-2 with 7% of anthesis and INDIRA KANKODA-1 with 5% of anthesis and during the period of 06:15 to 06:45 in the genotype RMDSG-3 recorded maximum 10% of anthesis followed by AJSG-1 with 8% of anthesis and AMBIKA 13-5 and INDIRA KANKODA-1 with 5% of anthesis.

Anthesis in most of the crosses genotypes was started during the period of 04:45 to 05:15. During the period of 04:15 to 04:45 the genotype AMBIKA 13-6 x AJSG-2 and AJSG-1 x CHHATTISGARH KANKODA-2 was showed maximum 40% anthesis followed by RMDSG-3 x CHHATTISGARH KANKODA-2 with 39% and NDM-2 x CHHATTISGARH KANKODA-2 with 38% anthesis and during the period of 04:45 to 05:15 in the genotype AMBIKA 13-5 x INDIRA KANKODA-1 recorded maximum 49% of anthesis followed by AMBIKA13-6 x AJSG-2 with 47% of anthesis and RMDSG-3 x INDIRA KANKODA-1 with 45% anthesis and during the period of 05:15 to 05:45 in the genotype NMD-2 x AJSG-2 recorded maximum 30% of anthesis followed by RMDSG-3 x CHHATTISGARH KANKODA-2, AMBIKA 13-5 x AJSG-2, AMBIKA13-6 x CHHATTISGARH KANKODA-2, NDM-5 x INDIRA KANKODA-1 and NDM-5 x CHHATTISGARH KANKODA-2 with 20% of anthesis and during the period of 05:45 to 06:15 in the genotypes AJSG-1 x INDIRA KANKODA-1, AJSG-1 x CHHATTISGARH KANKODA-2, AJSG-1 x AJSG-2, RMDSG-4 x INDIRA KANKODA-1, RMDSG-4 x CHHATTISGARH KANKODA-2, AMBIKA13-5 x CHHATTISGARH KANKODA-2, NDM-2 x INDIRA KANKODA-1, NDM-2 x CHHATTISGARH KANKODA-2, and NDM-5 x AJSG-2 recorded maximum 10% of anthesis followed by RMDSG-3 x CHHATTISGARH KANKODA-2, with 9% of anthesis and RMDSG-3 x INDIRA KANKODA-1 with 8% of anthesis and during the period of 06:15 to 06:45 in the genotypes RMDSG-3 x AJSG-2 and RMDSG-4 x AJSG-2 recorded maximum 10% of anthesis followed by AJSG-1 x INDIRA KANKODA-1 with 8% of anthesis and AMBIKA 13-5 x CHHATTISGARH KANKODA-2 with 7% anthesis and AJSG-1 x CHHATTISGARH KANKODA-2 with 6% of anthesis. It is observed that period of 4:45 AM to 5: 15 AM shoed the maximum dehiscence, so this period of time can be maximum utilized for the selection, artificial hybridization and to develop the new required varieties.

Receptivity of the Stigma before flowering and after flowering

In the study of stigma receptivity, the percentage of fruit was taken as an index (table 1 and 2). Artificial pollination by hand immediately after the opening of flowers results in 100% fruit set. The genotypes are a parent in AMBIKA13-6 are at anthesis time in 100% fruit set, the female flowers pollinated within 12 hours showed 69% fruit setting or 12 hours after anthesis also produced about 20% fruit set. Pollination before 14 hours of anthesis 45% fruit setting and after 14 hours old pollen result 5% fruit set in spine gourd genotype which indicates that stigma became receptive.

Followed by the genotypes of parent in NDM-2 are the female flowers pollinated within 12 hours showed 64% fruit setting or after 12 hours of anthesis also produced about 19% fruit set. Pollination before 14 hours of anthesis caused 54% fruit setting. And after 14 hours, old pollen result 7% fruit set in spine gourd genotype indicates that stigma became receptive. The genotype RMDSG-4 at anthesis time in 100% fruit set, pollination before 12 hours of anthesis caused 61% fruit set, and 12 to 18 hours before pollination less than 61% fruit set. And after 12 hours pollination caused 16% fruit set, at anthesis to 6 hours Pollination after 100% fruit set. And genotype AJSG-1 at anthesis time in 100% fruit set. Pollination after 10 hours anthesis result 35% fruit set, and 12 to 18 hours after anthesis result less than 65% fruit set.

The genotypes are crosses in CHHATTISGARH KANKODA-2 x AMBIKA13-6 are at anthesis time in 100% fruit set, the female flowers pollinated within 12 hours before 68% or 12 hours after anthesis also produced about 25% caused fruit set. Pollination before 14 hours of anthesis 58% with 14 hours after old pollen result 7% fruit set in spine gourd genotype indicates that stigma became receptive. Followed by the genotypes are crosses in CHHATTISGARH KANKODA-2 x NDM-5 are the female flowers pollinated within 12 hours before 70% or 12 hours after anthesis also produced about 20% caused fruit set. Pollination before 14 hours of anthesis 54% with 14 hours after old pollen result 5% fruit set in spine gourd genotype indicates that stigma became receptive. The genotype AJSG-2 x AMBIKA13-6 at anthesis time in 100% fruit set, pollination before 12 hours of anthesis in 61% fruit set, and 12 to 18 hours before pollination less than 61% fruit set, and after 12 hours pollination 27% fruit set. At anthesis to 6 hours Pollination after 100% fruit set. And genotype INDIRA KANKODA-1 x AMBIKA13-6 at anthesis time in 100% fruit set. In case of Pollination 12 hours before anthesis in 69% fruit set, and 12 to 18 hours before

pollination less than 69% fruit set, and 12 hours after pollination 5% fruit set. Since some fruit set was obtained from 18 hours old female flowers after anthesis was mainly due to the high viability of the fresh pollen.

Hence, failure of fruit set in the former case was certainly due to that the pollen grains were nonfunctional i.e. nonviable. Therefore, the studies indicated that the pollen became mature 2 hours before anther dehiscence and remain viable up to 18 hours after anthesis.

Although all female flowers pollinated within 12 h before or after anthesis produced fruit, it appears best to hand pollinate immediately after the opening of flowers to achieve 100% fruit set. Bud pollination, which is sometimes useful in research work may also, is successfully done within 12 h prior to anthesis.

Similarly reported from this result it reveals that for successful fruit set about 100% pollination should be done within 10 hours after anthesis. Bud pollination which sometimes becomes very useful in research work may also be successfully done 5 hours prior to anthesis, Similarly reported that the female plants, the stigma became receptive 18.5 h before anthesis, in 'Assam Local' it became receptive 18 h before anthesis. Hand pollination of buds 12 h before anthesis could set 60% of fruit in all female plants. However, hand pollination from the time of anthesis to 12 h after anthesis produced 100% fruit set in all- female clones. Receptivity of the stigma continued, although in declining mode, up to 26 h after anthesis in four plants, but in 'Assam Local' application of 12 hours old pollen to stigmas 12 hours after anthesis did not because fruit set Naik *et al.*, (2013)^[9].

Similarly reported in a pointed gourd that stigma remained receptive from 7 hours opening till 51 hours after opening. A shorter stigma receptivity period in the present study may be due to genotypic or *morphotypic* variation or may be due to agro-ecological variation also Pathak and Singh (1950)^[10].

Table 1: Crosses

S. No	Parents	S. No.	Crosses/Genotypes
1	AJSG-1	17	AJSG-1X INDIRA KANKODA-1
2	RMDSG-3	18	AJSG-1 X Chhattisgarh kankoda-2
3	RMDSG-4	19	AJSG-1 X AJSG-2
4	AMBIKA13-5	20	RMDSG-3 X INDIRA KANKODA-1
5	AMBIKA13-6	21	RMDSG-3X Chhattisgarh kankoda-2
6	NDM-2	22	RMDSG-3X AJSG-2
7	NDM-5	23	RMDSG-4 X INDIRA KANKODA-1
8	INDIRA KANKODA-1	24	RMDSG-4 X Chhattisgarh kankoda-2
9	Chhattisgarh kankoda-2	25	RMDSG-4 X AJSG-2
10	AJSG-2	26	AMBIKA13-5 X INDIRA KANKODA-1
11	AMBIKA13-5 X AMBIKA 12-1	27	NDM-2 X AJSG-2
12	AMBIKA13-5 X AJSG-2	28	NDM-5 X INDIRA KANKODA-1
13	AMBIKA13-6 X INDIRA KANKODA-1	29	NDM-5 X Chhattisgarh kankoda-2
14	AMBIKA13-6 X Chhattisgarh kankoda-2	30	NDM-5 X AJSG-2
15	AMBIKA13-6 X AJSG-2	31	NDM-2 X Chhattisgarh kankoda-2
16	NDM-2 X INDIRA KANKODA-1		

Table 2: Anthesis in the percentage of opened flower at the different time in a parent

Percentage of opened flower at a different time					
Genotype/Time	4:15 - 4:45	4:45-5:15	5:15- 5:45	5:45- 6:15	6:15- 6:45
AJSG-1	37	35	10	10	8
RMDSG-3	36	34	13	7	10
RMDSG-4	31	39	16	10	4
AMBIKA13-5	27	43	20	5	5
AMBIKA13-6	40	45	10	3	2
NDM-2	31	39	20	7	3
NDM-5	30	35	30	3	2
INDIRA KANKODA-1	32	48	10	5	5
CHHATTISGARH KANKODA-2	30	50	8	10	2
AJSG-2	33	37	17	10	3
Average	32.7	40.5	15.4	7	4.4

Table 3: Anthesis in the percentage of opened flower at a different time in crosses

Percentage of opened flower at a different time					
Genotype/Time	4:15 - 4:45	4:45-5:15	5:15- 5:45	5:45- 6:15	6:15- 6:45
AJSG-1 x INDIRA KANKODA-1	37	35	10	10	8
AJSG-1 x CHHATTISGARH KANKODA-2	40	30	14	10	6
AJSG-1 x AJSG-2	35	40	10	10	5
RMDSG-3 x INDIRA KANKODA-1	30	45	12	8	5
RMDSG-3 x CHHATTISGARH KANKODA-2	39	31	20	9	1
RMDSG-3 x AJSG-2	36	34	13	7	10
RMDSG-4 x INDIRA KANKODA-1	31	39	16	10	4
RMDSG-4 x CHHATTISGARH KANKODA-2	33	37	17	10	3
RMDSG-4 x AJSG-2	34	36	12	8	10
AMBIKA13-5 x INDIRA KANKODA-1	31	49	15	3	2
AMBIKA13-5 x CHHATTISGARH KANKODA-2	28	42	13	10	7
AMBIKA13-5 x AJSG-2	27	43	20	5	5
AMBIKA13-6 x INDIRA KANKODA-1	37	43	10	6	4
AMBIKA13-6 x CHHATTISGARH KANKODA-2	31	39	20	7	3
AMBIKA13-6 x AJSG-2	40	47	7	3	3
NDM-2 x INDIRA KANKODA-1	30	40	15	10	5
NDM-2 x CHHATTISGARH KANKODA-2	38	38	12	10	2
NDM-2 x AJSG-2	30	35	30	3	2
NDM-5 x INDIRA KANKODA-1	31	39	20	6	4
NDM-5 x CHHATTISGARH KANKODA-2	28	42	20	7	3
NDM-5 x AJSG-2	33	37	17	10	3
Average	33.29	39.10	15.38	7.71	4.52

Table 4: Stigma receptivity at different stages in parent

Before anthesis and after anthesis	Ajsg-1	Rmdsg-3	Rmdsg-4	Ambika13-5	Ambika13-6	NDM-2	NDM-5	Indira Kankoda-1	Chhattisgarh KANKODA-2	AJSG-2
Stage of bud / open flower										
18 hrs	21	18	17	14	20	11	15	20	17	15
16 hrs	28	30	31	31	33	34	30	31	30	36
14 hrs	50	39	48	45	45	54	47	41	42	43
12 hrs	65	60	61	55	69	64	56	62	63	63
At anthesis	100	100	100	100	100	100	100	100	100	100
2 hrs	100	100	100	100	100	100	100	100	100	100
4 hrs	100	100	100	100	100	100	100	100	100	100
6hrs	100	100	100	100	100	100	100	100	100	100
10 hrs	35	36	45	31	30	30	38	37	34	36
12hrs	25	15	16	19	20	19	10	11	17	13
14 hrs	5	7	5	7	5	7	5	5	5	7
16 hrs	4	8	7	9	7	9	7	5	5	9
18 hrs	5	7	9	10	12	10	5	6	4	7
Mean fruit setting%	49.08	47.69	49.15	47.77	49.31	49.08	47.15	47.54	47.46	48.38

Table 5: Stigma receptivity at different stages in crosses

Percentage of fruit set and advance to maturity in female plants																					
Before anthesis and after anthesis	Indira kankoda-1 x ajsg-1	Ik1 x rmdsg-3	Indira kankoda-1 x rmdsg-4	Indira kankoda-1 x ambika13-5	Indira kankoda-1 x ambika13-6	Indira kankoda-1 x ndm-2	Indira kankoda-1 x ndm-5	Chhattisgarh kankoda-2 x ajsg-1	Chhattisgarh kankoda-2 x rmdsg-3	Chhattisgarh kankoda-2 x rmdsg-4	Chhattisgarh kankoda-2 x ambika13-5	Chhattisgarh kankoda-2 x ambika13-6	Chhattisgarh kankoda-2 x ndm-2	Chhattisgarh kankoda-2 x ndm-5	Ajsg-2 x ajsg-1	Ajsg-2 x rmdsg-3	Ajsg-2 x rmdsg-4	Ajsg-2 x ambika13-5	Ajsg-2 x ambika13-6	Ajsg-2 x ndm-2	Ajsg-2 x ndm-5
Stage of bud /open flower																					
20hrs	5	7	9	10	12	10	5	6	4	7	5	8	4	5	4	5	4	4	4	7	7
18 hrs	21	18	17	14	20	11	15	20	17	15	19	17	14	19	20	14	15	12	21	11	19
16 hrs	28	30	31	31	33	34	30	31	30	36	35	39	30	31	35	32	30	36	37	30	31
14 hrs	50	39	48	45	45	54	47	41	42	43	54	58	49	54	50	49	47	45	45	55	48
12 hrs	65	60	61	55	69	64	56	62	63	63	69	68	67	70	74	60	74	60	61	64	65
At anthesis	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
2 hrs	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
4hrs	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
6 hrs	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
10 hrs	35	36	45	31	30	30	38	37	34	36	30	45	42	41	30	32	30	31	39	30	33
12 hrs	25	15	16	19	20	19	10	11	17	13	20	25	25	20	20	21	26	23	27	21	20
14 hrs	5	7	5	7	5	7	5	5	5	7	7	7	5	5	5	3	4	7	3	2	2
16 hrs	3	5	2	4	6	7	2	3	4	6	2	8	5	6	4	2	5	6	7	5	4
22 hrs	3	5	2	4	6	7	2	3	4	6	2	8	5	6	4	2	5	6	7	5	4
21 hrs	5	7	5	7	5	7	5	5	5	7	7	7	5	5	5	3	4	7	3	2	2
Mean fruit setting%	43.00	41.93	42.73	41.80	43.40	43.33	41.00	41.60	41.67	42.60	43.33	46.00	43.40	44.13	43.40	41.53	42.93	42.47	43.60	42.13	42.33

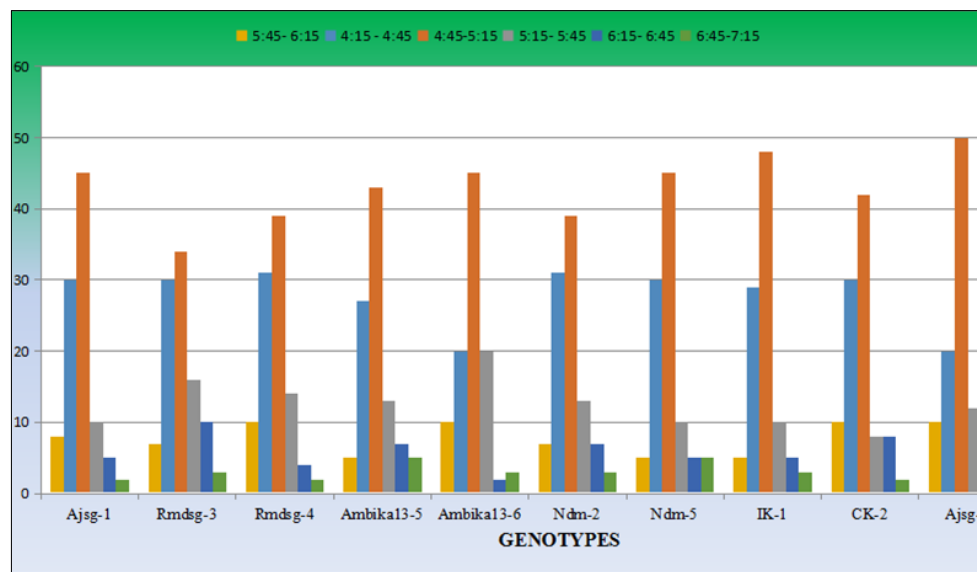


Fig 1: Percentage of anthesis in flower opened at the different time in parent

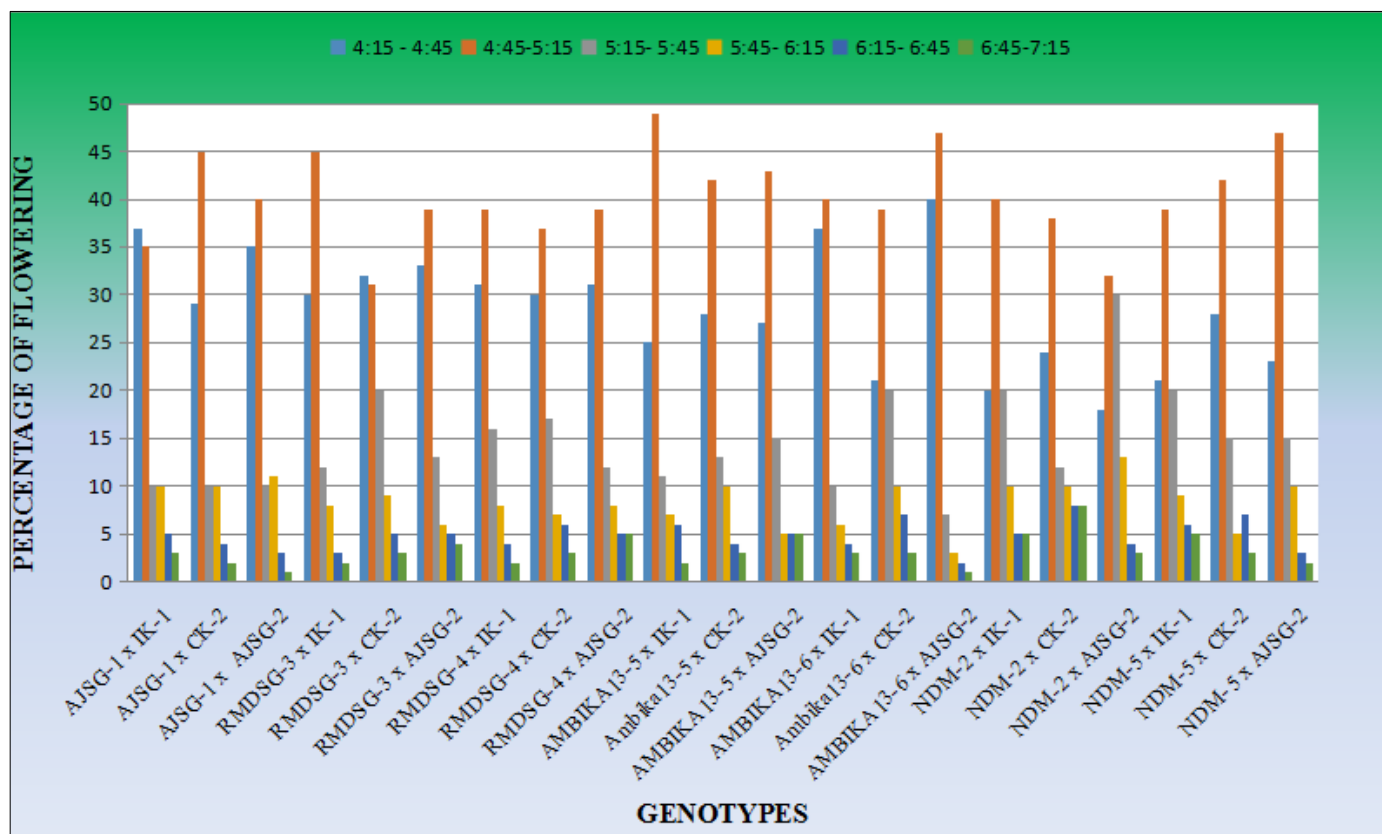


Fig 2: Percentage of anthesis in flower opened at the different time in crosses

Conclusion

Anthesis of most of the genotypes was started from 04:15 to 05:15 am and maximum receptivity of stigma was observed during the period of at anthesis to 12 hrs after anthesis.

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