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Reproductive biology of *Citrus aurantifolia* CV Kuliana Lime under east and south east coastal plain zone of Odisha

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Abstract
Acid lime being a tropical fruit crop, it's flushing and flowering depends on the variation of climatic factors. Kuliana lime is an elite species of Odisha. An experiment was conducted on the reproductive behaviour of 4 year old air layered Kuliana lime in three seasons (Rainy season, winter season and spring season) at Horticultural Research Station, Department of Fruit Science and Horticulture Technology, O.U.A.T., Bhubaneswar during 2016-17. The data clearly revealed that the spring season was superior with respect to reproductive shoot (90.41%), axillary bud (93.15%), hermaphrodite flower (18.45%) and pollen viability (77.42%), which indicates the potential scope for improving the cultivar by adopting breeding technologies as well cultural aspect while taking the bahar that favour the spring season flowering.

Keywords: acid lime, *Citrus aurantifolia* Swingle, reproductive biology, floral behaviour, pollen viability, axillary bud, days to anthesis and kuliana lime.

Introduction

Citrus (*Citrus* sp.) is the World's leading tree-fruit crop which positioned as third most important fruit crop in India next to Mango and Banana. It occupies a place of considerable importance in the fruit economy of the country. Acid Lime *(Citrus aurantifolia* Swingle), from the family Rutaceae originated in India and then spread to the Middle East and other tropical and subtropical countries (Salunkhe and Desai, 1984) [24]. The major citrus producing countries are Brazil, Spain, USA, Israel, Morocco, China, Mexico, Russia, India, Canada, South Africa etc. In India, it is cultivated in 1.042 Mha area with the production of 10.089 MT, among which lemon and lime contribute about 0.255 Mha in area, 2.523 MT on production basis and the major producing states are Maharastra, Andhra Pradesh, Punjab etc (NHB database, 2014).

Odisha accounts for 2.4% and ranked 9th to the national production of citrus fruits. Mayurbhanj, Keonjhar, Koraput, Ganjam, Gajapati, Dhenkanal are the major lime growing areas in Odisha. The area and production of lime and lemon is 26.63 thousand ha and 261.07 thousand tonnes respectively in Odisha (Ministry of Agriculture, Horticulture Statistics at a Glance 2015). Kuliana lime is a local elite land race of Mayurbhanj district, Odisha, where it is extensively grown as the hot summer and cold winter climate of the area is highly suitable for its cultivation. This local land race is traditionally grown in village kuliana from which the name has come as Kuliana lime and is widely popular in the state for its size and juice content. Since the lime plant has a wide range of soil and climatic adaptation, it is necessary to improve this fruit crop through planned breeding. Hence as a prerequisite, it is essential to study the floral biology of the fruit crop as this will provides the useful information regarding the period of anthesis, anther dehiscence and stigmatic receptivity.

Materials and Methods

A field experiment was conducted on 4 year old air layered acid lime cultivar Kuliana lime planted at a spacing of 4×4 m in Horticultural Research Station, Department of Fruit Science and Horticulture Technology, O.U.A.T., Bhubaneswar during 2016-17. The experimental site comes under the eighteenth agro climatic region of the country i.e. "Eastern Coastal Plain'' and is termed as sub humid. The climate here is warm, humid with distinct summer, rainy and winter seasons. The present experiment was conducted in "Random Effect Model under PROC MIXED through Statistical Analysis System (SAS), software with three treatment (season) and ten replication (plant). The various intercultural operations like irrigation, manuring weeding etc. are done according to the recommendation for acid lime plant. Plant protection measures were taken as and when required to control the seasonal pest and disease incident. Different characters on floral behaviour including reproductive shoot blooming season, duration of flowering, days to anthesis, blooming habit (terminally or in the axil), types of flower (hermaphrodite and staminate flowers) and viability of pollen were recorded. The pollen grains from freshly opened flowers were mounded on clean glass slides and examined under microscope. The shape and colour of pollen grain were recorded. Pollen fertility of both hermaphrodite and staminate flower are studied by acetocarmine test. All the pollen grain which took pink stain after two hours are considered as fertile one and others as sterile.

Results and Discussion

Reproductive shoot

All the three seasons were having significant Influence on conversion of vegetative shoot to reproductive shoot (Table-1). In spring season maximum (90.41%) vegetative shoot of previous season growth were converted to the reproductive shoot by producing flower buds and it was followed by the winter season (73.74%). The lowest (64.1%) conversion was obtained in the rainy season. Spring season has positive impact in increasing the conversion of vegetative shoot to reproductive shoot at P<0.0001 over the rainy season (10.9%) and winter season (4.88%). The impact of winter season was greater (1.16%) in conversion of vegetative shoot to reproductive shoot than that of the rainy season. The time of initiation and differentiation varies by citrus cultivar and environmental conditions (Abbott, 1935) [1]. Webber (1943) [29] observed that the time of flowering was reported to vary with temperature. Flowering was about a month earlier in higher temperature zone (Florida) than in cooler temperature zone (California). When the hours of low temperature increases with a period of water stress bud sprouting shifted from the vegetative bud (no flowers) to mixed bud (both flowers and leaves) or reproductive bud (leafless at least one flower). The winter low temperature condition causes the inactivity of buds while inducing them to flower in *Citrus.* Maximum flowering occurred only during December - January and May - July periods as greater accumulation of hours of low temperature increases floral induction. The result was in line with Cassin *et al*. (1969); Moss (1969); Reuther *et al*. (1973); Hittalamani (1977); Hittalamani *et al*. (1977); Poerwanto and Inoue (1990); Garcialuis *et al*. (1995); Guardiola (1997); Srivastava *et al*. (2000) and Kumatkar *et al*. (2016). [3, 19, 22, 21, 9, 25, 14] The number of spring shoots is determined by internal factors connected with previous crop load and the extent of vegetative flushes prior to bud sprouting in spring (Goldschmidt and Monselise, 1972) [8]. Bud age and location in the tree canopy have a definite influence on sprouting and flowering (Krajewski and Rabe, 1995a) [13]. Mishra *et al*. (2018) [17] observed spring season was best in production of new growth flush or tertiary shoots which bear flower in latter part of the season.

Duration of flowering

Data pertaining to duration of flowering presented on Table-1 was found significantly different among the three seasons. The average duration of flowering was extended to 58.00 days (20/12/2016 to 15/02/2017) in spring season followed by the rainy season (50.00 days) from 24/04/2016 to 13/06/2016 and minimum (45.00 days) was recorded in the winter season from 01/09/2016 to 15/10/2016. All the seasons showed significant Influence on the average duration of flowering. Spring season over winter season and rainy season recorded an increase of 13 days and 8 days in the average duration of flowering (P<0.0001 each) and in rainy season over winter season it was 5 days (P<0.0001). Duration of extension of growth and flowering were greatly accelerated or slowed by climatic conditions. The less number of flowering days in the winter season may be attributed to the less diurnal variation in temperature and lowered relative humidity with higher bright sunshine hour during the flowering period. The spring season recorded the maximum duration of flowering which may be due to the low temperature at the time of flowering in December to February. Similar findings were observed by Cooper *et al*. (1963); Rohidas and Chakrawar (1989); Moss (1973); Valiente and Albrigo (2002) and Tripathi and Dhakal (2005) [4, 23, 18, 28, 27].

Days to anthesis

Data from Table-1 shows significant variation on total days to anthesis in Kuliana lime. The rainy season flowering takes maximum days (11.08 days) from flower bud initiation to anthesis followed by the winter season (9.01 days) and spring season (9.0 days). Winter season and spring season were statistically at par with each other. Rainy season over spring and winter season recorded an increase of 2.08 and 2.07 days in the average days taken for anthesis in Kuliana lime with a P<0.0001 each. Favourable climatic condition enhances the differentiation hence increasing the cellular growth and reduces the growth period. Sustained periods of low temperature in winter caused late anthesis by delaying initiation of differentiation and reducing the subsequent growth rate of the bud. The observation is similar to Guardiola (1997); Moss (1973); Lomas and Burd (1983); Bellows and Morse (1986) and Thirugnanavel *et al.* (2017) [9, 13, 15, 2, 17].

Axillary buds and terminal buds

It was evident from Table-2 that, in Kuliana lime there is no significant difference in the percentage of axillary buds among the three seasons. However the spring season recorded the maximum (93.15%) number of axillary buds followed by the rainy season (92.89%) and the minimum (92.55%) percentage of axillary buds were seen in the winter season flowering. In case of terminal bud percentage the same situation also persists. In the winter season the terminal bud percentage was more (7.47%) followed by the rainy season (7.23%) and the spring season recorded the minimum (6.84%) percentage of terminal flower (Table-2). All the three seasons have similar influence in production of the terminal and axillary buds and in Kuliana lime and the flowering mostly observed in the axillary buds. Similar findings were obtained by Hittalamani (1977) and Hittalamani *et al*. (1977) who reported that *Citrus aurantifolia* Swingle mainly bore flowers in lateral or axillary shoot, whereas *C. latifolia* flowered on terminal shoots. Bud behavior in spring depends critically both on the time the buds were formed (bud age) and in the position of the bud along the shoot (Guardiola, 1997) [9]. The shoots developed during the flushes of growth arise mainly from the axillary buds present in the vegetative shoots formed in previous flushes of growth (Devi *et al.* (2011); Karmakar (2013) [5, 12].

Staminate and Hermaphrodite flower

Rainy season recorded the maximum (84.99%) per cent of staminate flower followed by the winter season (82.49%) and the minimum (81.52%) was in the spring season (Table-2). All the three season have significant Influence in the production of staminate flowers. Among the seasons the rainy season recorded an increase in production of staminate flower of 0.27 per cent and 0.14 per cent over the spring and winter season respectively (P<0.0001 each), whereas the winter season shows 0.01 per cent over the spring season (P= 0.0005). Spring season recorded the maximum (18.45%) hermaphrodite flower followed by the winter season (15.2%) and the minimum (15%) was observed in the rainy season (Table-2). The percentage of hermaphrodite flower produced in the spring season were 0.21 per cent and 0.01 per cent more than that of the rainy season (P<0.0001) and winter season (P=0.0064) respectively and in the winter season there was a increase of 0.17 per cent of production of hermaphrodite flower in Kuliana lime over the rainy season. The percentage of hermaphrodite flowers was highest during spring season and flowering was related to the season rather than to the physiological age of the shoots. The findings were not in line with Hittalmani (1977) and Hittalmani *et al.* (1977) [10]. Higher temperature during rainy season flowering might be the reason behind the lower hermaphrodite flower in that particular season (Geetha *et al*., 2016) [7].

Pollen viability of staminate and hermaphrodite flower

The pollen viability data of staminate flower on percentage (Table-3 and Figure-1) of total pollen illustrated significant difference among the seasons. The percentage of viable pollen in staminate flower during the spring season (77.42%) was significantly superior to the winter season (75.64%) and the lowest was observed in the rainy season (70.09%). There was no significant variation of pollen viability of staminate flower in rainy season and winter season but spring season recorded an increase of 0.026 per cent and 0.046 per cent over the rainy season (P=0.0025) and winter season (P=0.0002) respectively. The pollen viability data of hermaphrodite flower on percentage (Table-3 and Figure-2) of total pollen illustrated significant difference among the seasons. Highest percent of viable pollen in the hermaphrodite flower was obtained in rainy season (75.17%) which is at par with the spring season (74.21%) and the lowest was in the winter season (70.22%). The rainy season and spring season have similar Influence in pollen viability of hermaphrodite flower. Rainy season over winter season (P=0.0133) followed by the spring season over winter season (P=0.0403) have impact in increasing the pollen viability of Kuliana lime. The increase in the pollen viability of Kuliana lime in spring season might be attributed to the suitable climatic condition during the early part of the season.

Conclusion

From the experiment conducted it can be concluded that spring season flowering was superior than the other seasons with respect to conversion of one season old vegetative shoot to reproductive shoot, duration of flowering, production of hermaphrodite flower, pollen viability etc. To achieve good yield in Kuliana lime bahar treatment must be imposed to encourage spring season flowering. Crop improvement work can also be taken up during spring season flowering to achieve success and production of more number of seeds. Hence the retention of spring season flowers by manipulation of cultural practices may be advised for the growers of Kuliana lime.

Table 1: Influence of season on the reproductive shoot, duration of flowering and the days to anthesis of Kuliana lime

Season	Plant	Reproductive shoot (%)	Duration of flowering (days)	Days to anthesis
Rainy	1	66.5 (54.63)	50	11
	2	61.4 (51.59)	52	10.2
	3	70.4 (57.04)	49	10
	4	65.2 (53.85)	51	12.2
	5	57.6 (49.37)	50	10.68
	6	65.5 (54.03)	49	11.49
	7	54.6 (47.64)	50	11.98
	8	65 (53.73)	51	12
	9	71.4 (57.67)	48	9.8
	10	62.9 (52.48)	50	11.47
	Mean	64.1 (53.20)	50	11.08
Winter	1	76.3 (60.87)	45	9
	2	79.6 (63.15)	46	9.09
	3	62.4 (52.18)	45	9.46
	4	82.4 (65.2)	47	9.2
	5	71.7 (57.86)	45	9
	6	80.2 (63.58)	44	8.92
	7	63.5 (52.83)	45	9.02
	8	69.5 (56.48)	43	9
	9	77.5 (61.68)	45	9.01
	10	72.9 (58.63)	45	8.4
	Mean	73.74 (59.25)	45	9.01
Spring	1	90.6 (72.15)	57	9.46
	2	87.5 (69.3)	56	9.03
	3	95.2 (77.34)	59	8.91
	4	95 (77.08)	55	9.1
	5	91.3 (72.84)	60	8.9
	6	90.4 (71.95)	58	9
	7	88.3 (70)	61	9.12
	8	82.9 (65.57)	59	8.27
	9	90.2 (71.76)	58	9.16
	10	90.5 (72.05)	57	9.05
	Mean	90.41 (72.00)	58	9

SE (±) Season		1.1764	0.4389	0.1765
SEd (±) of LSM		1.5004	0.6206	0.2497
Pr >|t|	Rainy spring	<0.0001	<0.0001	<0.0001
	Rainy winter	0.0008	<0.0001	<0.0001
	Spring winter	<0.0001	<0.0001	NS

Figure in the parenthesis denotes Arc Sine value

Table 2: Influence of season on production of axillay bud, terminal bud, staminate flower and hermaphrodite flower in Kuliana lime

Season	Plant	Axillary bud (%)	Terminal bud (%)	Staminate flower per plant (%)	Hermaphrodite flower per plant (%)
Rainy	1	93.2 (74.88)	6.8 (15.12)	85 (67.21)	15 (22.79)
	2	94 (75.82)	6 (14.18)	84.6 (66.89)	15.4 (23.11)
	3	91.56 (73.11)	8.44 (16.89)	85.1 (67.29)	14.9 (22.71)
	4	90.56 (72.11)	9.44 (17.89)	84.35 (66.7)	15.65 (23.3)
	5	94.58 (76.54)	5.42 (13.45)	85.6 (67.7)	14.4 (22.3)
	6	92.9 (74.55)	7.1 (15.45)	84.89 (67.05)	15.2 (22.95)
	7	93.56 (75.29)	6.44 (14.7)	85.89 (67.86)	14.2 (22.14)
	8	92.58 (74.19)	7.42 (15.81)	84.8 (67.05)	15.2 (22.95)
	9	92.56 (74.17)	7.44 (15.83)	84.9 (67.13)	15.1 (22.87)
	10	92.1 (73.57)	7.9 (16.32)	84.9 (67.13)	15.1 (22.87)
	Mean	92.89 (74.42)	7.23 (15.56)	84.99 (67.20)	15 (22.79)
Winter	1	93 (74.66)	7 (15.34)	82.6 (65.35)	17.4 (24.65)
	2	91.7 (73.26)	8.3 (16.74)	82.6 (65.35)	17.4 (24.65)
	3	92.9 (74.55)	7.1 (15.45)	81.6 (65.35)	18.4 (25.4)
	4	92.46 (74.07)	7.54 (15.94)	82.7 (65.42)	17.3 (24.58)
	5	93.76 (75.55)	6.24 (14.47)	82.5 (65.27)	17.5 (24.73)
	6	92.56 (74.17)	7.44 (15.83)	81.2 (64.3)	18.8 (25.7)
	7	91.3 (72.84)	8.7 (17.16)	81.8 (64.75)	18.2 (25.25)
	8	92.6 (74.21)	7.4 (15.79)	83.4 (65.96)	16.6 (24.04)
	9	92.6 (74.21)	7.4 (15.79)	83.4 (65.96)	16.6 (24.04)
	10	92.52 (74.13)	7.48 (15.87)	82 (64.9)	18 (25.1)
	Mean	92.55 (74.16)	7.47 (15.84)	82.49 (65.26)	15.02 (24.81)
Spring	1	94.7 (76.69)	5.3 (13.31)	81.9 (64.92)	18.1 (25.18)
	2	93.2 (74.88)	6.8 (15.12)	81.2 (64.3)	18.8 (25.7)
	3	94.5 (76.44)	5.5 (13.56)	81.8 (64.75)	18.2 (25.25)
	4	91.98 (73.55)	8.02 (16.45)	81.2 (64.3)	18.8 (25.7)
	5	91.7 (73.26)	8.3 (16.74)	82.8 (64.75)	17.2 (24.5)
	6	94.3 (76.19)	5.7 (13.81)	81.2 (64.3)	18.8 (25.7)
	7	93.4 (75.11)	6.6 (14.89)	81.1 (64.23)	18.9 (25.77)
	8	93.1 (74.77)	6.9 (15.23)	81.3 (64.38)	18.7 (25.62)
	9	91.97 (73.54)	8.03 (16.47)	81.7 (64.67)	18.3 (25.33)
	10	92.35 (73.93)	7.65 (16.05)	81.75 (64.71)	18.25 (25.29)
	Mean	93.15 (74.83)	6.84 (15.16)	81.52(64.53)	18.45(25.40)
SE (±) Season		0.359	0.358	0.1218	0.1383
SEd (±) of LSM		0.5077	0.5064	0.1714	0.1911
Pr >|t|	Rainy spring	NS	NS	<0.0001	<0.0001
	Rainy winter	NS	NS	<0.0001	<0.0001
	Spring winter	NS	NS	0.0005	0.0064

Figure in the parenthesis denotes Arc Sine value

Table 3: Influence of season on pollen viability of Kuliana lime

Season	Plants	Pollen viability (%)	
		Staminate flower	Hermaphrodite flower
Rainy	1	77.02 (60.68)	72.34 (58.27)
	2	75.98 (60.66)	73.54 (59.03)
	3	76.96 (60.95)	71.65 (57.83)
	4	76.12 (60.74)	72.96 (58.67)
	5	76.84 (61.23)	72.5 (58.37)
	6	73.42 (58.96)	73.2 (58.82)
	7	76.2 (60.8)	72.4 (58.31)
	8	74.56 (59.71)	71.98 (72.52)
	9	75.56 (60.37)	73.2 (58.82)
	10	79.54 (63.1)	75.83 (60.54)
	Mean	70.09 (60.72)	75.17 (60.12)
Winter	1	75.8 (60.53)	69.5 (56.48)
	2	74.92 (59.94)	69.8 (56.66)
	3	76.32 (60.88)	70.82 (57.29)
	4	75.65 (60.435)	70.24 (56.93)
	5	75.86 (60.57)	71.2 (57.54)

	6	74.32 (59.55)	71.34 (57.63)
	7	75.92 (60.61)	71 (57.42)
	8	75.56 (60.37)	68.7 (55.98)
	9	76.1 (60.73)	69.8 (56.66)
	10	76.05 (60.7)	70 (56.79)
	Mean	75.64 (60.43)	70.22 (56.94)
Spring	1	77.32 (61.56)	75.44 (60.29)
	2	77.44 (61.64)	74.24 (59.5)
	3	77.98 (62.02)	73.12 (58.76)
	4	77.86 (61.93)	74.86 (59.9)
	5	77.21 (61.48)	74.52 (59.68)
	6	77.01 (61.34)	73.35 (58.9)
	7	76.98 (61.33)	73.2 (58.82)
	8	77.79 (61.88)	74.26 (59.51)
	9	77.43 (61.63)	73.98 (59.33)
	10	77.38 (61.61)	75.43 (60.280)
	Mean	77.42 (61.64)	74.21 (59.49)
SE (±) Season		0.2124	0.8186
SEd (±) of LSM		0.2623	1.1577
Pr >|t|	Rainy spring	0.0025	NS
	Rainy winter	NS	0.0133
	Spring winter	0.0002	0.0403

Figure in the parenthesis denotes Arc Sine value)

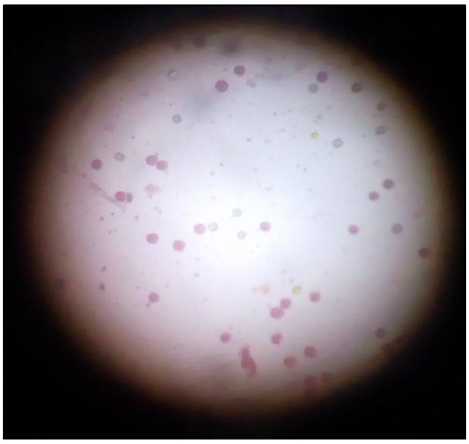

Fig 1: Pollen viability of staminate flower of Kuliana lime

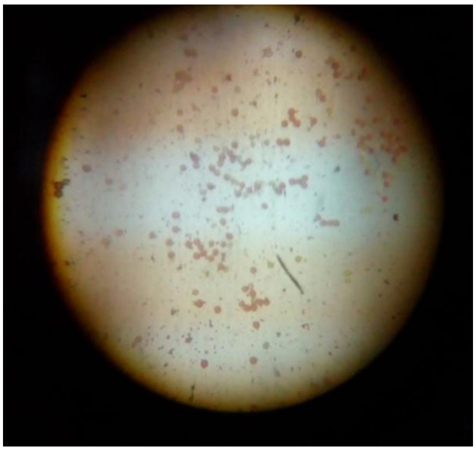

Fig 2: Pollen viability of hermaphrodite flower of Kuliana lime

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