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ASSESSMENT OF POLLEN VIABILITY AND FLORAL BIOLOGY IN SWEET ORANGE (*CITRUS SINENSIS* OBSECK) CULTIVARS UNDER SUBTROPICAL CONDITIONS OF PUNJAB

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ABSTRACT
Assessment of pollen viability is an essential tool to check the level of sterility as it limits sexual hybridization. The percentage of viable pollen of 20 sweet orange cultivars was evaluated. The viability percentage values ranged from 5.0% for 'New Hall Navel' orange to 80.0% for 'Mosambi' sweet orange. The 'Mosambi' sweet orange cultivar showed the highest percentage of viable pollen, while viable pollen were absent in navel oranges. The cultivars 'New Hall Navel', 'Cara Cara Navel' and 'Washington Navel', sweet orange cultivars, showed the lowest percentage of viable pollen (5.00%, 5.50% and 5.75% respectively). Among flower characters, cultivar Campbell Valencia recorded with highest flower diameter and flower length (41.21 mm and 29.67 mm, respectively) while petal length and petal width was recorded maximum in Campbell Valencia and Cutter Valencia (23.65 mm and 9.34 mm, respectively).

INTRODUCTION

Citrus is the most widely produced fruit group in the world. Abudant heterozygosis is present in most citrus form. Breeding in citrus is much complicated by heterozygosity, hence information on pollen viability and floral morphology are the ideal tools to select a superior genotype with better cross combination for hybridization work and Till date, however, very limited work on assessment of pollen viability and floral morphology among sweet orange has been carried out under sub-tropics of india. Various test procedure have been carried out to determine pollen viability in fruit trees (Norton, 1966; Heslop-Harrison and Heslop- Harrison, 1970; Parfitt and Ganeshan, 1989). Germination test can be considered as more reliable way to determine exact amount of viable pollen (Bolat and Pirlak, 1999). Viability tests will provide a better idea to select of promising cultivar among several, which can be further used in hybridization program as a pollen parent. The objective of this study was to determine the pollen viability and floral biology to assess a cultivar with high pollen viability percentage, greater cross combination and with greater fruit set.

MATERIALS AND METHODS

Fresh pollens grains of 20 cultivars of sweet orange namely Campbell Valencia, Cara Cara Navel, Crescent Orange, Cutter Valencia, Delta Valencia, Early Gold, Fukumoto Navel, Itaborai, Jaffa, Moro, Mosambi, New Hall Navel, Olinda Valencia, Rhode Red Valencia, Ruby Nucellar, Sanguinelli, Trovita, Vernia, Washington Navel and Westin from uniformly managed plants growing at College Orchard and Fruit Research Farm of Punjab Agricultural University, Ludhiana during the year 2014 and 2015 respectively. Pollens collected during the spring (February-March) of 2014 and 2015 were used in the experiment. In order to determine the pollen viability, well-grown 20 flowers from each variety were picked about at ten o'clock in the balloon. They were put in paper bags and were brought to the laboratory. To determine the viable capacity of pollen, 1% of acetocarmine stain solution was determined. One or two drops of acetocarmine solution was put on a clean micro slide and kept for 5-10 min. at ambient conditions (Norton 1966). For this assay, two lamella for each cultivars and four regions of each lamella were investigated; viable, semi-viable and dead pollen numbers and their percentages were determined. Pollen viability was scored based on the staining level as pollen with red color viable, with light red semi-viable and with colorless nonviable.

Floral morphological characters was determined for the characters such as flower diameter, flower length, petal length, petal width, calyx diameter, length of filament, length of style, pedicel length by using Digital Varnier Calipers (Mituyoto Inc., Japan) on the basis of IPGRI (International Plant Genetic Resources Institute) citrus descriptors, Rome, Italy (Anonymous 1999).

Data were analyzed statistically to an analysis of variance (ANOVA) and differences among the means were determined for significance at $p < 0.05$ by LSD test using the statistical analysis system software version 9.3 (SAS Institute Inc., Cary,

NC, USA) at 5% level of probability. Mean and standard errors of each sample were calculated for statistical comparison (Singh *et al*., 1998).

RESULTS AND DISCUSSION

The pollen viability in sweet orange cultivars was found to be significantly different in the acetocarmine solution (Table 1). The viable pollen recorded as highest was obtained from the anthers of Mosambi (80.00%) followed by Campbell Valencia (67.50%), while the lowest viable pollen was obtained from the anthers of New Hall Navel (5.00%) in the acetocarmine stain test. It is clear from the following results that a cultivar with higher pollen viability percentage such as Mosambi and Campbell Valencia can be used as a doner parent in hybridization program, while a cultivar with lower pollen viabililty percentage such as New Hall Navel, Washington Navel and Cara Cara Navel have non viable pollen hence can't be used in varietal improvement or hybridization program. In a similar investigation, variations in pollen viability was also reported by Demir *et al* (2015) who found that the maximum pollen viability was recorded in Meyer variety of lemon (86.74), while the minimum pollen viability was in Batem Sarisi lemon (40.62). Similarly, Ilgin M *et al* (2007) also reported pollen viability of five capri fig (*Ficus cariaca*) genotypes using triphenyltetrazolium chloride (TTC) and fluroscent diacetat (FDA) and found that the percentage of viable pollen in caprifig types ranged from 76.04 to 83.34% by TTC test and from 75.60 to 86.73% by FDA test.

Table 1: Pollen viability percentage among sweet orange cultivars in acetocarmine stain

Cultivars	Pollen viability (%)
Campbell Valencia	67.50 [b]
Cara Cara Navel	5.50 [i]
Crescent Orange	62.25 [bc]
Cutter Valencia	36.25 [fg]
Delta Valenica	40.75 [ef]
Early Gold	29.50 [gh]
Fukumoto Navel	55.50 [cd]
Itaborai	48.25 [de]
Jaffa	62.50 [bc]
Moro	29.50 [gh]
Mosambi	80.00 [a]
New Hall Navel	5.00 [i]
Olinda Valencia	25.50 [h]
Rhode Red Valencia	42.25 [ef]
Ruby Nucellar	66.75 [b]
Sanguinelli	43.50 [ef]
Trovita	64.50 [b]
Vernia	65.00 [b]
Washington Navel	5.75 [i]
Westin	65.75 [b]
Mean	45.07
LSD (pd"0.05)	8.86

Different alphabets shows significant difference and same alphabets shows non significant difference among cultivars

Venkateshwarlu and Lavania (1985) in lemon observed that the pollen viability ranged from 78.04 (Florida rough) to 91.66% (Nepali Oblong) among the seven cultivars studied. Pollen viability was recorded 83.34% in Seedless lemon (Rohidas and Chakarwar, 1982).

Floral morphological characters were found to be significantly different among all the sweet orange varieties (Table 2, 3 and 4 respectively). Flowering initiation and full bloom period was the earliest in variety Early Gold (6th March and 18th – 25th March, respectively), while termination of flowering season was commenced firstly in Ruby Nucellar (30th March). Staminate flower percentage was noted highest in Crescent Orange (15%), however, the highest perfect flower percentage was recorded in Moro (94.50%). Flower diameter and flower length was recorded maximum in Campbell Valencia (41.21 mm and 29.67 mm, respectively), while the minimum flower diameter and flower length was recorded in Sanguinelli and Trovita (25.69 mm and 16.14 mm, respectively). Petal length and petal width was the highest in Campbell Valencia and Cutter Valencia (23.65 mm and 9.34 mm, respectively), while both these observation were recorded as lowest in Trovita

Table 2: Flowering period among sweet orange cultivars

Varieties	Start date of flowering	Full bloom date of flowering	End date of flowering
Campbell Valencia	13th March	24th -26th March	2nd April
Cara Cara Navel	13th March	24th - 27th March	2nd April
Crescent Orange	14th March	24th - 27th March	3rd April
Cutter Valencia	12th March	25th -28th March	2nd April
Delta Valenica	15th March	23rd -26th March	3rd April
Early Gold	6th March	18th - 23rd March	1st April
Fukumoto Navel	12th March	21st -25th March	2nd April
Itaborai	8th March	20th - 23rd March	3rd April
Jaffa	14th March	22nd - 24th March	2ndApril
Moro	20th March	25th - 29th March	2nd April
Mosambi	8th March	18th - 25th March	2nd April
New Hall Navel	14th March	23rd - 27th March	2nd April
Olinda Valencia	15th March	26th - 29th March	3rd April
Rhode Red Valencia	11th March	22nd - 26th March	1st April
Ruby Nucellar	9th March	21st - 24th March	30th March
Sanguinelli	12th March	22nd - 25th March	31st March
Trovita	10th March	20th - 25th March	31st March
Vernia	14th March	24th - 27th March	1st April
Washington Navel	14th March	25th -28th March	4th April
Westin	10th March	21st - 23rd March	31st March

Table 3: Floral morphological characters among sweet orange cultivars

Varieties	Flower diameter (mm)	Flower length (mm)	Staminate flower (%)	Perfect flower (%)	Pedicel length (mm)
Campbell Valencia	41.21^{a}	29.67^{a}	9.25 ^{bcde}	90.75 ^{bcde}	8.89^{b}
Cara Cara Navel	32.07^{defg}	20.85^{hij}	6.50 ^{ef}	93.50 ^{ab}	6.38^{l}
Crescent Orange	32.90^{cdefg}	21.92^{fghi}	15.00 ^{a}	85.25 ^{f}	6.15^{o}
Cutter Valencia	34.80^{cde}	26.51^{b}	10.50 ^{bc}	89.50 ^{de}	9.81^{a}
Delta Valenica	35.55^{bcd}	22.71^{efgh}	10.00 ^{bcd}	90.00 ^{cde}	7.87^{f}
Early Gold	34.69^{cdef}	20.21^{ijk}	9.75 ^{bcd}	90.25 ^{cde}	6.86^{h}
Fukumoto Navel	33.56^{cdef}	21.22^{ghij}	11.25 ^{b}	88.75 ^{e}	6.66^{j}
Itaborai	32.58^{cdefg}	24.09^{cde}	10.25 ^{bc}	89.75 ^{de}	6.75^{i}
Jaffa	36.25^{bc}	24.73^{bcd}	10.00 ^{bcd}	90.00 ^{cde}	8.89^{b}
Moro	30.83^{efgh}	21.37^{ghi}	5.50 ^{f}	94.50 ^{a}	7.86^{f}
Mosambi	39.01^{ab}	24.28^{cde}	6.75 ^{ef}	93.25 ^{ab}	7.75^{g}
New Hall Navel	32.19^{defg}	20.59^{ij}	7.25 ^{def}	92.75 ^{abc}	6.29^{m}
Olinda Valencia	36.35^{bc}	25.30^{bc}	10.00 ^{bcd}	90.00 ^{cde}	8.74^{c}
Rhode Red Valencia	34.95^{cd}	22.95^{defg}	11.25 ^{b}	88.75 ^{e}	8.24^{e}
Ruby Nucellar	31.93^{defgh}	19.30^{jkl}	8.25 ^{cdef}	91.75 ^{abcd}	5.35^{q}
Sanguinelli	25.69^{i}	17.03^{mn}	10.25 ^{bc}	89.75 ^{de}	6.63^{k}
Trovita	27.98^{hi}	16.14^{n}	11.25 ^{b}	88.75 ^{f}	5.84^{p}
Vernia	29.52^{ghi}	18.51^{klm}	9.25 ^{bcde}	90.75 ^{bcde}	6.24^{n}
Washington Navel	35.20^{bcd}	23.42^{cdef}	8.50^{bcde}	91.50 ^{bcde}	8.50^{d}
Westin	30.76^{fgh}	18.10^{lmn}	6.75 ^{ef}	93.25 ^{ab}	6.85^{h}
Mean	33.40	21.94	9.37	90.63	7.32
LSD (pd″0.05)	4.02	2.00	2.83	2.80	0.03

Different alphabets shows significant difference and same alphabets shows non significant difference among cultivars

Table 4: Floral morphological traits among sweet orange cultivars.

Varieties	Length of filament (mm)	Length of style (mm)	Number of stamens	Petal length (mm)	Petal width (mm)
Campbell Valencia	11.36^{a}	11.08^{a}	23.00^{a}	23.65^{a}	8.44^{abcd}
Cara Cara Navel	9.03^{i}	4.67^{m}	22.50^{abc}	15.49^{gh}	7.63^{defg}
Crescent Orange	10.91^{d}	6.32^{g}	21.25^{def}	17.42^{ef}	6.57^{hijk}
Cutter Valencia	8.79^{k}	6.85^{d}	22.50^{abc}	20.40^{b}	9.34^{a}
Delta Valenica	10.51^{e}	6.83^{d}	21.75^{bcde}	19.97^{bc}	7.47^{defgh}
Early Gold	8.36^{m}	5.84^{h}	22.25^{abcd}	17.72^{def}	8.80^{ab}
Fukumoto Navel	8.22^{o}	3.56^{q}	21.75^{bcde}	15.49^{gh}	6.98^{ghij}
Itaborai	8.82^{j}	5.69^{i}	20.50^{f}	18.68^{cde}	8.06^{bcdef}
Jaffa	10.90^{d}	6.37^{f}	21.75^{bcde}	19.45^{bc}	7.93^{cdefg}
Moro	10.11^{g}	6.86^{d}	21.75^{bcde}	17.98^{def}	5.63^{k}
Mosambi	11.13^{b}	6.90^{c}	21.00^{ef}	19.09^{bcd}	8.38^{abcde}
New Hall Navel	8.49^{l}	7.14^{b}	22.75^{ab}	15.66^{gh}	8.95^{ab}
Olinda Valencia	10.38^{f}	6.60^{e}	22.25^{abcd}	19.68^{bc}	8.62^{abc}
Rhode Red Valencia	8.17^{p}	3.67^{p}	21.75^{bcde}	19.44^{bc}	7.15^{fghi}
Ruby Nucellar	8.34^{n}	4.75^{l}	21.50^{cdef}	17.14^{f}	7.53^{defgh}
Sanguinelli	6.18^{s}	4.60^{n}	22.75^{ab}	14.90^{h}	6.06^{jk}
Trovita	7.65^{r}	2.95^{r}	22.50^{abc}	14.41^{h}	7.43^{efghi}
Vernia	7.87^{q}	4.51^{o}	21.00^{ef}	15.31^{gh}	6.44^{ijk}
Washington Navel	10.95^{c}	5.04^{j}	22.50^{abc}	17.52^{ef}	7.41^{efghi}
Westin	9.28^{h}	4.80^{k}	22.50^{abc}	16.63^{fg}	7.12^{fghi}
Mean	9.27	5.75	21.97	17.80	7.60
LSD (pd″0.05)	0.02	0.03	1.16	1.42	0.98

Different alphabets shows significant difference and same alphabets shows non significant difference among cultivars

and Moro (14.41 mm and 5.63 mm, respectively). Pedicel length was maximum in Cutter Valencia (9.81 mm) and was minimum in Ruby Nucellar (5.35 mm). Length of filament and style was the maximum in Campbell Valencia (11.36 mm and 11.08 mm, respectively), while the lowest length of filament and style was observed in Sanguinelli and Trovita (6.18 mm and 2.95 mm, respectively). Number of stamens per flower was recorded in variety Campbell Valencia (23.00), while the lowest number of stamen was there in Itaborai (20.50). Rathod *et al* (2014) studied floral and reproductive phenology in Aloe vera while, Sharma *et al* (2013) also characterized flower biology in bottle guard. In a similar study in six varieties of lemon Singh and Shanker (1964) also concluded that the highest staminate flowers (68.4%) were recorded in Nepali oblong followed by Lucknow Seedless (61.0%) and Hill lemon (57.1%). Kinley and Chinawat (2011) reported that petal length was the maximum in Samtse and Zhemgang mandarin (11.3 mm each) and was the minimum in Tsirang and Dagana mandarin (10.9 mm each), while petal width was the maximum in Sarpang, Tsirang, Trongsa and Zhemgang (4.8 each) and

the minimum was in Samtse and Dagana mandarin (4.7 mm each). Similarly, number of stamens was the maximum in Tsirang and Dagana mandarin (14.9 each) and it was the minimum in Samtse mandarin (14.6).

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