

STUDIES ON FRUITING AND FRUIT QUALITY CHARACTERISTICS IN TISSUE CULTURED PLANTS OF POMEGRANATE CV. BHAGWA

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ABSTRACT

A study was undertaken to investigate the possible effects of different chemicals (Nitrobenzene (NB) @ 1.0 ml plant⁻¹, NB @ 1.5 ml plant⁻¹, NB @ 2.0 ml plant⁻¹, Cycocel (CCC) @ 500 ppm plant⁻¹, CCC @ 1000 ppm plant⁻¹, CCC @ 1500 ppm plant⁻¹, Uracil @ 25 ppm plant⁻¹, Uracil @ 50 ppm plant⁻¹, CCC @ 1000 ppm plant⁻¹ + Uracil @ 25 ppm plant⁻¹, CCC @ 1500 ppm plant⁻¹ + Uracil @ 50 ppm plant⁻¹) on fruiting, fruit quality characteristics concomitant with anthocyanin content in pomegranate cv Bhagwa during the years 2016 – 17 at ICAR – IIHR, Hesaraghatta. The results showed that the fruits with highest weight (197.55 g), length (6.64 cm), total aril weight (113.63 g), percentage of aril weight (62.11 %), 100 aril weight (27.18 g), juice weight (101.94 g) and percentage (51.19 %) accompanied with highest anthocyanin content (6.647 mg 100g⁻¹) were obtained with application of CCC @ 1000 ppm plant⁻¹ in combination with Uracil @ 25 ppm. Highest fruit diameter (6.90 cm), TSS content (19.96 °B), TSS to acid ratio (46.68) and less titrable acidity (0.41 %) were apparent with foliar spray of Cycocel @ 1500 ppm plant⁻¹, while its application @ 1000 ppm has resulted in highest fruit volume (162.70 ml).

KEYWORDS: Pomegranate, Fruit Quality & Tissue Cultured

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INTRODUCTION

Pomegranate (*Punica granatum* L.) is a member of Punicaceae (Syn. Lythraceae) family, bearing 2n = 16 or 18 number of chromosomes. Globally, India is having the largest area under pomegranate cultivation, as well as the largest producer. It produces 25.21 million tonnes of pomegranate, from an area of 0.21 m. ha (Anon, 2017). The popularity of the crop and its cultivation is gaining impetus in arid and semi-arid regions of India, could be attributed to its wide range of adoptability, its ability to grow even on marginal and low fertile soils, to withstand hot and hostile climate, tolerance to alkaline and saline soils, low maintenance cost and high remunerative returns per unit area along with its medicinal properties owing to presence of polyphenols like punicalgins in outer rind and ellagic acid, in arils and anthocyanins such as delphinidin, cyanidin and pelargonidin which possesses antioxidant potential. The unique plasticity of pomegranate is apparent from the threshold limit it exhibits for higher (44°C) and lower -12°C temperature (Westwood, 1978).

There are three main flowering seasons in pomegranate i.e. *Ambe bahar*, *Mrig bahar* and *Hastha bahar*. The selection of a particular bahar at a location is mainly determined by prevailing production factors like availability of irrigation water, marketing factors, occurrence of disease and insect – pests, otherwise uninterrupted continuous blossom would likely produce a light fruit crop throughout the year and requires a high cost of cultivation (Singh *et al.* 1967). *Ambe bahar* in northern India is preferred due to profuse flowering, hot and dry climate during fruit development stages, whereas, the fruit crop obtained during *Mrig bahar* is severely damaged by a higher incidence of insect – pests and diseases as a result of monsoon showers. Thus, continuous and uninterrupted flowering in *Ambe bahar*, for a long period significantly produces fruit of various grades, which in turn adversely affects the overall fruit development, fruit quality and net marketable returns (Sharma and Nav Prem, 2002). Pomegranate fruit has a good consumer preference for its attractive, juicy, sweet acidic and refreshing arils. There is a growing demand for good quality fruits both for fresh use and processed products (Pruthi and Saxena, 1984). Cycocel, a growth retardant, which acts as an antigibberellin compound and arrests the vegetative bud development, nucleic acid synthesis and protein metabolism, by specific anti – metabolites, which induce flower formation, as well as enhances fruit quality (Nir *et al.* 1972).

The present study was conducted on tissue cultured plants of pomegranate cv. Bhagwa, using chemicals like Nitrobenzene, Cycocel and Uracil applied at different concentrations litre⁻¹ plant⁻¹ prior to full bloom stage to analyze the fruiting and fruit quality parameters viz., fruit weight, fruit length, diameter and volume, total aril weight, 100 aril weight and percentage of aril weight along with quality parameters viz., Total soluble solids (TSS), Titrable acidity, TSS / Acid ratio, Juice percentage and Anthocyanin content after harvesting of fruits.

MATERIALS AND METHODS

The present study was conducted on healthy and uniformly grown tissue culture plants of pomegranate cv Bhagwa, procured from M/S Jain Irrigation Pvt Ltd, Jalgoan (Maharashtra) at the ICAR- Indian Institute of Horticultural Research, Hesaraghatta farm, Bengaluru during *Ambe bahar* (January to February) season of the years 2016-17. ICAR - IIHR is situated at an altitude of 890 meter above mean sea level at 13° 7' North latitude and 77° 29' East longitudes, respectively. The maximum and minimum temperatures during the experiment were 33.08°C and 20.43°C, and relative humidity and rainfall recorded were 75.04% and 74.95 mm, respectively. The experiment consisted of eleven treatments which were replicated thrice and the statistical design used was Randomized Block Design (RBD). The treatments included were T₁ – Nitrobenzene (NB) @ 1.0 ml litre⁻¹ plant⁻¹, T₂ – NB @ 1.5 ml litre⁻¹ plant⁻¹, T₃ – NB @ 2.0 ml litre⁻¹ plant⁻¹, T₄ – Cycocel (CCC) @ 500 ppm litre⁻¹ plant⁻¹, T₅ – CCC @ 1000 ppm litre⁻¹ plant⁻¹, T₆ – CCC @ 1500 ppm litre⁻¹ plant⁻¹, T₇ – Uracil @ 25 ppm litre⁻¹ plant⁻¹, T₈ – Uracil @ 50 ppm litre⁻¹ plant⁻¹, T₉ – T₅ + T₇, T₁₀ – T₆ + T₈ and T₁₁ – Control.

After harvesting of fruits, observations were manifested on fruit weight, fruit volume, fruit length, fruit diameter, total aril weight, 100 aril weight, percentage of aril weight, Total soluble solids (TSS), Titrable acidity, Juice weight, juice percentage and anthocyanin content. For recording these parameters, three fruits were selected randomly from each replication of respective treatment.

Fruit Weight

Individual fruit weight was measured in randomly selected nine fruits in each treatment and the mean value was expressed in grams (g fruit⁻¹).

Fruit Length

The maximum length of the individual fruit from stalk to styler end was measured in cm with the help of digital vernier calipers and the mean fruit length was expressed in centimeters (cm).

Fruit Diameter

The maximum diameter of each fruit was measured diagonally using digital vernier calipers and the mean value was calculated and expressed as centimeters (cm).

Fruit Volume

The volume of fruits was measured by water displacement method and the mean value was calculated and expressed as ml.

Total Aril Weight

The arils were separated from the randomly selected nine fruits under each treatment and weighed individually. The mean aril weight was expressed in grams (g fruit^{-1}).

Hundred Aril weight

The weight of 100 arils was recorded by extracting the 100 arils from each fruit among the nine randomly selected fruits in each treatment and the mean value was expressed in grams.

Percentage of Aril Weight

The percentage of aril weight was calculated by using the formula:

$$\frac{\text{Aril weight (g)} \times 100}{\text{Fruit weight (g)}}$$

Juice Percentage

The juice percentage was calculated by using the formula:

$$\frac{\text{Juice weight (g)} \times 100}{\text{Fruit weight}}$$

Total Soluble Solids

TSS content of the fruit was quantified by using Carl – Zeiss Hand held refractometer and the mean value was expressed in $^{\circ}\text{Brix}$ (Ranganna, 1986).

Titration Acidity

Acidity was estimated by titration method (AOAC, 2000). Ten grams juice was measured in a measuring cylinder and the juice collected was made up to the volume of 50 ml with distilled water. 10 ml of this filtrate was titrated against 0.01N NaOH, using Phenolphthalein as indicator. Acidity was calculated as mg of citric acid equivalents per 100 g fresh weight using citric acid standard curve.

TSS to Acid Ratio

TSS to Acid ratio was obtained by dividing TSS with titrable acidity.

Anthocyanin Content

The anthocyanin content in fruits was determined as per the method described by Ranganna (1986). Anthocyanin was extracted from the sample by blending 10 g of sample with 10 ml of ethanolic HCL and transferred to 100 ml volumetric flask. The volume was made up and the solution was stored in refrigerator at 4°C, and then filtered through Whatman No.1 filter paper. Optical density of filtrate was recorded at 520 nm.

Anthocyanin (mg / 100g) =

$\frac{O.D_{520} \times \text{volume made up} \times 100}{\text{Weight of sample (g)}}$

Weight of sample (g)

Statistical Analysis

The data was analyzed as per the method of variance, outlined by Panse and Sukhatme (1985). Statistical significance was tested by F value at 5% level of significance. Critical difference at 0.05 levels was worked out for the effects, which were significant.

RESULTS AND DISCUSSIONS

Fruiting Parameters

It is conspicuous from Table 1 that, all the treatments were influenced significantly pertaining to fruiting parameters. Highest fruit weight (197.55 g), fruit length (6.64 cm), total aril weight (113.63 g), percentage of aril weight (62.11 %) and 100 aril weight (27.18 g) were apparent with application of Cycocel @ 1000 ppm in combination with Uracil @ 25 ppm. Application of Cycocel @ 1500 ppm has enhanced fruit diameter (6.90 cm), while its application @ 1000 ppm resulted in increased fruit volume (162.70 ml). The increase in fruit weight with Cycocel could be attributed to its inhibitory action by exhibiting anti – gibberellin response and possible diversion of photosynthates, for flowering and fruiting (Guha, 1993 and Mansuroglu *et al.* 2009). The maximum fruit size in terms of length and diameter due to Cycocel application might be due to increased level of carbohydrate and Cycocel might have stimulated cell division and cell elongation, resulting in larger fruit size, as reported by Singh and Phogat (1984) and Thakur *et al.* (1990) in litchi. The reason for increasing fruit volume with Cycocel might be due to increase in the level of fruit size. The result is in conformity with the earlier report, by Suryanarayan and Dass (1971) in litchi and Pandey *et al.* (2001) in guava.

Fruit Quality Characteristics

Significantly, highest juice weight (101.94 g) and percentage (51.19 %) were registered with application of Cycocel @ 1000 ppm combined with Uracil @ 25 ppm. Application of Cycocel @ 1500 ppm displayed significantly highest TSS content (19.96 °B), TSS to acid ratio and less titrable acidity (0.41 %) (Table 2). The increase in the juice appears to be due to translocation of sugars and water in the arils. With the rise in water content of arils, the percentage of seed had naturally declined in proportion to the juice content. Similar results were in concurrence with Supe and Saitwal (2016). The increase in total soluble solids were might be due to the metabolizing effect of growth retardant (Cycocel) and their effect on osmotic pressure of the cells tends to increase and solutes like ions and sugars accumulates and thus the TSS

level was increased in treated fruits (Singh, 1996). The increase in TSS could also be attributed to hydrolysis of starch into sugars. The similar results were reported by Joshi and Roy (1985). The increased TSS to acid ratio might be due to increase in total soluble solids content and reduction in acid content of the fruits. The improvement in fruit quality with the application of growth retardants might be due to diversion of photosynthates, towards the fruits (Rai and Bist, 1992).

Anthocyanin Content

Application of Cycocel @ 1000 ppm in combination with Uracil @ 25 ppm has recorded significantly highest anthocyanin content in fruit juice (6.467 mg 100g⁻¹). The increase in the total amount of anthocyanins with the application of Cycocel may be due to the continued biosynthesis of phenolic compounds after harvest, related to the ripening process. The increase in anthocyanin concentration after harvest was correlated with the activity of enzymes of the anthocyanin biosynthetic pathway: Phenylalanine Ammonia Lyase (PAL) and UDP – glucose, flavonoid – 3 – O – glucosyl transferase (GT). Similar results were in agreement with Gil *et al.* (1995).

CONCLUSIONS

In this paper, it has been reported about the effects of Cycocel in influencing the fruiting and fruit quality characteristics concomitant, with Anthocyanin content. Application of Cycocel @ 1000 ppm in combination with Uracil @ 25 ppm and Cycocel @ 1500 ppm, has enhanced the said parameters in tissue cultured plants of pomegranate cv. Bhagwa.

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Table 1: Fruiting Parameters in Pomegranate cv Bhagwa as Influenced by Chemicals

Treatments	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Fruit volume (ml)	Total aril weight (g)	Percentage of aril weight (%)	100 Aril weight (g)
T ₁ - NB @ 1.0 ml plant ⁻¹	177.80	6.34	6.49	136.58	95.73	54.76	24.92
T ₂ - NB @ 1.5 ml plant ⁻¹	187.07	6.47	6.74	125.53	103.94	55.31	24.42
T ₃ - NB @ 2.0 ml plant ⁻¹	183.00	6.18	6.38	143.26	97.30	53.20	24.65
T ₄ - CCC @ 500 ppm plant ⁻¹	196.31	6.57	6.83	160.08	110.80	58.23	26.97
T ₅ - CCC @ 1000 ppm plant ⁻¹	193.66	6.57	6.84	162.70	105.00	52.31	26.58
T ₆ - CCC @ 1500 ppm plant ⁻¹	187.25	6.50	6.90	155.62	104.84	56.16	25.87
T ₇ - Uracil @ 25 ppm plant ⁻¹	176.82	6.40	6.67	126.43	96.26	57.92	23.86
T ₈ - Uracil @ 50 ppm plant ⁻¹	176.88	6.37	6.75	125.37	98.55	59.16	24.74
T ₉ - T ₅ + T ₇	197.55	6.64	6.82	160.54	113.63	62.11	27.18
T ₁₀ - T ₆ + T ₈	187.43	6.62	6.59	150.60	97.05	56.05	24.50
T ₁₁ - Control	166.54	6.03	6.20	135.05	84.13	50.68	20.07
C.D. at 5%	15.66	0.39	0.30	27.81	14.63	7.20	2.78
S. Em (±)	5.27	0.13	0.10	9.36	4.92	2.44	0.93
CV %	4.94	3.59	2.62	11.27	8.47	7.55	6.52

*NB – Nitrobenzene and CCC – Cycocel

Table 2: The Effect of Different Chemicals on Fruit Quality Attributes of Pomegranate cv Bhagwa

Treatments	TSS (°B)	Titration acidity (%)	TSS / Acid ratio	Juice weight (g)	Juice percentage (%)
T ₁ - NB @ 1.0 ml plant ⁻¹	18.50	0.54	34.25	56.91	31.95
T ₂ - NB @ 1.5 ml plant ⁻¹	16.39	0.68	24.10	89.93	47.50
T ₃ - NB @ 2.0 ml plant ⁻¹	18.25	0.52	35.61	62.01	33.83
T ₄ - CCC @ 500 ppm plant ⁻¹	19.12	0.48	39.83	92.31	47.01
T ₅ - CCC @ 1000 ppm plant ⁻¹	19.44	0.43	45.20	86.66	44.63
T ₆ - CCC @ 1500 ppm plant ⁻¹	19.96	0.41	48.68	72.37	38.38
T ₇ - Uracil @ 25 ppm plant ⁻¹	15.82	0.66	23.96	60.73	34.33
T ₈ - Uracil @ 50 ppm plant ⁻¹	17.22	0.67	25.70	51.27	28.98
T ₉ - T ₅ + T ₇	16.07	0.69	23.28	101.94	51.19
T ₁₀ - T ₆ + T ₈	16.16	0.70	23.08	88.95	47.37

T₁₁ – Control	15.46	0.60	27.91	61.10	36.66
C.D. at 5%	1.54	0.17	10.28	25.87	10.76
S.Em (±)	0.51	0.05	3.46	8.71	3.62
CV %	5.13	17.56	18.71	20.13	15.62

Table 3: Anthocyanin Content in Pomegranate cv Bhagwa as Influenced by Different Chemicals

Treatments	Anthocyanin content (mg 100 g⁻¹)
T₁ – NB @ 1.0 ml plant⁻¹	5.077
T₂ – NB @ 1.5 ml plant⁻¹	5.147
T₃ – NB @ 2.0 ml plant⁻¹	5.273
T₄ – CCC @ 100 ppm plant⁻¹	5.607
T₅ – CCC @ 150 ppm plant⁻¹	5.803
T₆ – CCC @ 200 ppm plant⁻¹	5.110
T₇ – Uracil @ 25 ppm plant⁻¹	4.660
T₈ – Uracil @ 50 ppm plant⁻¹	4.847
T₉ – T₅ + T₇	6.467
T₁₀ – T₆ + T₈	4.810
T₁₁ – Control	3.557
C.D. at 5%	1.233
S.Em (±)	0.415
CV %	14.032

*NB – Nitrobenzene and CCC – Cycocel

