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Correlation and PCA studies in CPPU applied mango (*Mangifera indica* L.) cv. "Dusehri"

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Abstract
The present investigation aimed to study the correlation and principle component analysis of N1-(2-chloro-4-pyridyl)-N3-phenyl urea (CPPU) on fruit retention, yield, and quality of mango cv. Dusehri during 2019-20 and 2020-21 at Punjab Agricultural University, Ludhiana, India. The growth hormones promoting fruit retention such as CPPU (@ 5, 10, and 15 ppm), salicylic acid (@ 100, 200, and 300 ppm) and 2,4-dichlorophenoxyacetic acid (2,4-D) (@ 20 ppm), and were sprayed during pea stage of fruit growth. Fruit yield was significantly and positively associated with fruit retention at the marble and harvest stage, fruit length, fruit diameter, number of fruits per plant, fruit weight, fruit pulp weight, firmness, shelf life, fruit TSS, total sugar and TSS: acidity ratio. However, fruit yield was significantly and negatively correlated with fruit acidity and fruit cracking.

Keywords: Fruit retention, salicylic acid, cytokinin, fruit size, fruit cracking

1. Introduction
Improving plant productivity in modern agriculture increasingly depends on manipulating plant physiological activities through the use of chemicals. Fruit drop is a serious setback to mango production in all over the world including India. Mango (*Mangifera indica* L), the most popular fruit in many parts of the world, is grown in more than 100 countries in tropical and subtropical climates. It is the most exquisite fruit of India and rightly bears the title of "King of Fruits" and National Fruit of India due to its great adaptability, variety of varieties, delicious taste, excellent taste, nutritional value, attractive appearance and popularity among the masses. India is the largest mango producer in the world, accounting for 45.2% of global mango production, and the annual production is 20.89 million tons from an area of 2.31 million hectares (Anonymous, 2021) [1].

Regardless of good cropping area, the productivity of mango in India is low. Amongst the commercial producers of mangoes, Brazil (16.5 MT/ha) and Indonesia (12.6 MT/ha) has highest productivity than India (9.02 MT/ha). Thus, productivity improvement continues to be the foremost issue for mango production. Majority of the mango importing countries are considering India as a source of quality mangoes due to its diversity in varietal wealth and wide window of availability. Hence, collective efforts are necessary to improve the productivity as well as quality of Indian mangoes to achieve standards and enhance its availability for the domestic as well as international market (Balamohan and Devi 2014) [3].

Despite adequate flowering, excessive fruit drop is the prime factor accounting for low productivity in mango orchards and even less than 0.10% of set fruits reach the harvest stage (Chadha 1993) [7] causing a great economic loss in all mango production (Singh and Singh 1995; Malik and Singh 2006) [25, 16]. Some of the suggested reasons for fruit drop are lack of pollination, failure of fertilization, sink competition between fruits, self-incompatibility, embryo abortion, hormonal imbalance, nutrient deficiency, climatic factors, deficient soil moisture and less photosynthesis (Chadha 1993; Bains *et al.* 1997) [7, 2]. The major factors causing mango fruit drop are: a deficiency of auxins, gibberellins, and cytokinins and higher levels of inhibitors such as ethylene and abscisic acid (Krisanapook *et al.* 2000; Ram 2000) [11, 22]. Trials have been conducted in the past to improve fruit set and minimize fruit abscission in mango by various workers using auxins (2.4-D, NAA, and 2.4.5-T), cytokinin, gibberellins and growth retardants (cycocel and alar).

Exogenous application of Salicylic acid inhibits aminocyclopropane carboxylic acid (ACC) synthase, an enzyme that forms ethylene from ACC (Leslie and Romani 1986) [14]. Forchlorfenuron (CPPU) belongs to synthetic cytokinins group which is known to promote cell division and inhibit cytokinin oxidation in plants (Mok and Mok 2001) [17]. Exogenous application of CPPU improved fruit retention and yield in various mango cultivars and growing regions (Burondkar *et al.* 2009; Notodimedjo 2000) [6, 19]. Fruit retention and yield were enhanced by exogenous administration of CPPU and salicylic acid in a variety of mango cultivars and growth environments.

In light of the aforementioned details, the current inquiry "Correlation and PCA studies in CPPU applied mango (*Mangifera indica* L.) cv. "Dusehri" was planned to study the principle component analysis and correlation of various factors associated with fruit yield in response to CPPU treatments.

2. Materials and Methods

2.1 Experimental location

This study was conducted for two cropping seasons during 2019–20 (ON year) and 2020–21 (OFF year) in the Department of Fruit Science, Punjab Agricultural University (PAU), Ludhiana, Punjab, India. The experiment was carried out in two sites to neutralize the impact of the surrounding environment. Location 1 (Ladhowal) is located at University Seed Farm, Ladhowal, Ludhiana, Punjab, and represents the trans-gangetic alluvial plains of Punjab at 30.97°N latitude and 75.75°E longitude. Location-2: situated at M. S. Randhawa Fruit Research substation, Gangian (Hoshiarpur), represents a sub-mountainous zone at the foothills of the Shivalik mountain range, Punjab at 31.48°N latitude and 75.58°E longitude. The meteorological data of both the locations and cropping seasons (2019-20 and 2020-21) are given in Figure 1.

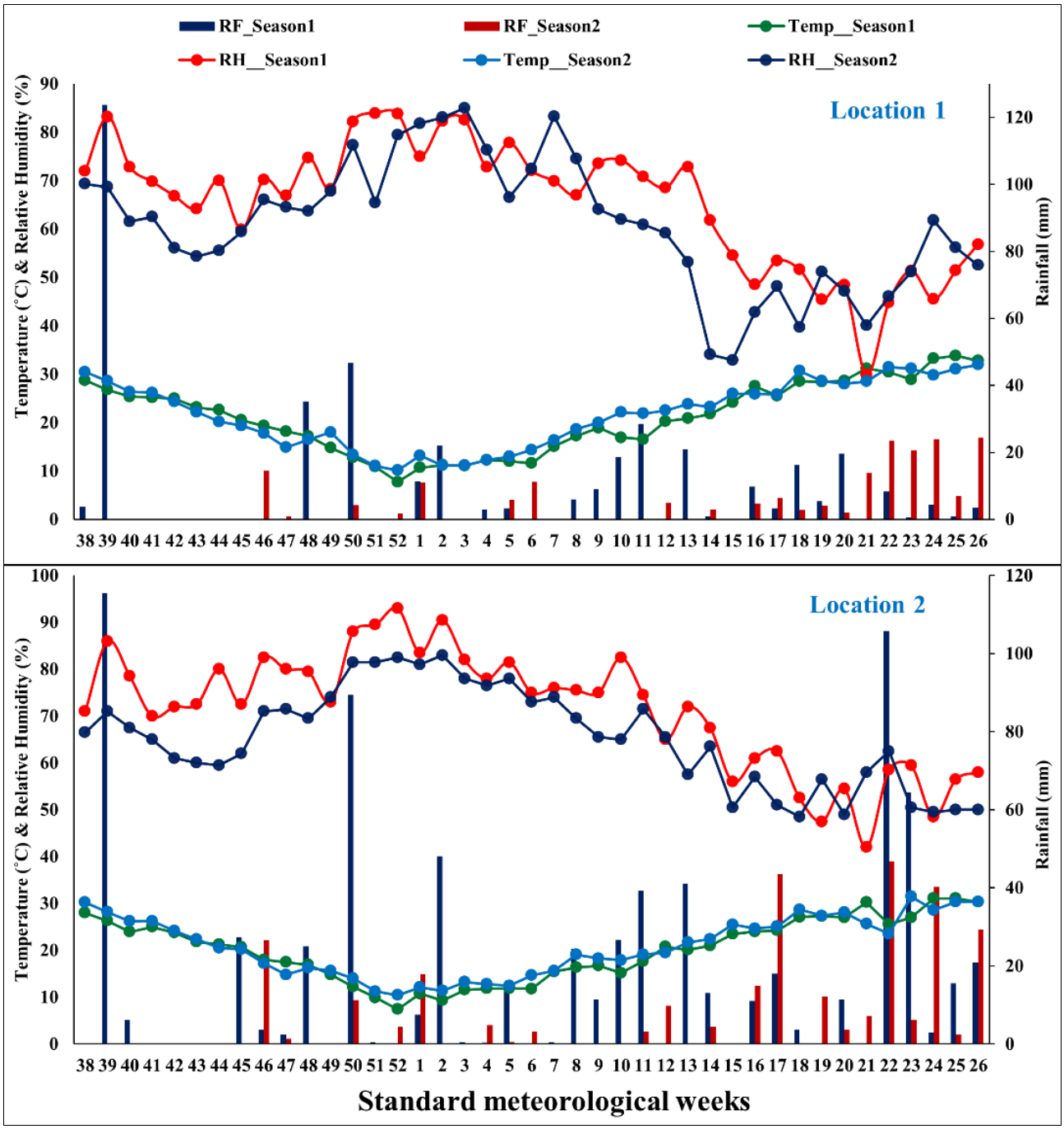

Fig 1: Weekly meteorological data of location 1 (Ladhowal) and location 2 (Gangian). (*Temp= Temperature, RF= Rain Fall; RH- Relative Humidity)

2.2 Experimental material and treatment details

The investigation was conducted on an approximately 25-year-old grafted mango cv. Dusehri kept a 10m x 10m space. During the trials, the plants were treated to standard uniform cultural methods and plant protection measures for mango growing as established by PAU. The varying concentrations of CPPU and SA were chosen based on previous research. The current recommendation of 2,4-D (20 ppm) was utilized as a baseline to compare the efficiency of CPPU and SA. The following is a list of the treatments used in this study: T1- 2.4.-D @ 20 ppm, T2-Salicylic acid @ 100 ppm, T3-Salicylic acid @ 200 ppm, T4-Salicylic acid @ 300 ppm, T5-CPPU @ 5 ppm, T6-CPPU @ 10 ppm, T7-CPPU @ 15 ppm and T8-Control.

2.3 Application of plant growth substances

Sitofex® (0.1% w/w; AlzChem) as CPPU, Salicylic acid (SA-99% by Thermo Fisher Scientific India Pvt. Ltd) and salt form of 2.4-D (Laboratory grade) were used for the study. Using a tractor-operated sprayer with a spray capacity of 15 liters per tree, the necessary concentration of all the growth regulators was sprayed on pre-selected consistently flowered trees during the pea stage of fruit growth. The spraying activities were conducted in the morning using Tween-80 as the surfactant.

2.4 Observations

2.4.1 Fruit retention

Before applying treatment, 40 terminal panicles of the same size were randomly marked in four directions for each tree while it was in full bloom. At the pea stage, the initial fruit set was determined by counting all of the fruitlets that were born on each panicle. Fruits retained on tagged panicles were counted at marble and harvest stages and the following formula was used to determine the fruit retention percentage.

$$\text{Fruit retention (\%)} = \frac{\text{Number of fruits @ the initial stage}}{\text{Number of fruits @ marble/ harvest stage}} \times 100$$

2.4.2 Fruit yield & quality attributes

Fruits that had reached physiological maturity were harvested and the fruit yield, length and diameter of fruits were recorded. Three-ply corrugated fiber board boxes with paper lining and 5% ventilation were filled with ten fruits from each treatment, each with three replications (30 fruits/treatment) and let to naturally ripen at room temperature. Total soluble solids (TSS) of ripen fruits was measured at 20 °C using an Erma Hand Refractometer (0–32 °Brix). The TSS: acid ratio was calculated by dividing TSS by acidity. The amount of total carotenoids was calculated using Jenson's (1978) method. From the marble to fruit maturity stage, the total number of cracked fruits was counted, and the incidence of fruit cracking was calculated using the formula below.

$$\text{Fruit cracking (\%)} = \frac{\text{No. of cracked fruits per plant}}{\text{Total no. of fruits per plant}} \times 100$$

2.5 Statistical analysis

The experiment was designed in a Randomized Block Design (RBD), with eight treatments and three replications, each treatment containing a single tree. The data was subjected to an analysis of variance (ANOVA) using the 'Agricolae' package that came with the 'R' software. The Fisher's Least Significant Difference (LSD) test was used to compare treatment means multiple times ($p \leq 0.05$). To determine the degree of relationship between associated features, the Pearson correlation coefficients were computed using the R package "Corrplot". Principal component analysis (PCA) was carried out with the "Factoextra" package included with R studio.

3. Result and Discussion

3.1 Correlation

Pearson correlation coefficient based on average values from the two seasons (2019-20 and 2020-21) for location 1 (Ladhowal) and location 2 (Gangian) are furnished in figure 2 and 3 respectively.

3.1.1 Fruit retention

The data from the results indicate that the fruit yield was significantly and positively associated with fruit retention at the marble stage (r = 0.83 in 1st location and r = 0.94 in 2nd location) and fruit retention at the harvest stage (r = 0.96 in 1st location and r = 0.98 in 2nd location). Findings of two cropping seasons at two locations demonstrated that, topical application of Salicylic acid and CPPU markedly improved fruit retention at marble and harvest stage over control with CPPU being the most effective. Under the current experiment, greater fruit set and enhanced fruit retention may be the result of CPPU's significant inhibition of cytokinin oxidation (Mok and Mok, 2001) [17] and promotion of cell division and expansion of mango fruit (Chen, 1983; Ram, 1983) [8, 23]. According to a recent study, CPPU can help grapes set berries by lowering respiration metabolism, maintaining higher energy charge levels through increased respiratory metabolic pathways such as the tricarboxylic acid (TCA) cycle and the Embden-Meyerhof pathway (EMP), and decreasing the pentose phosphate pathway (Yu *et al.*, 2021) [27]. The results of Pujari *et al.* (2016) [21], Bhamare *et al.* (2014) [4], Kulkarni *et al.* (2017) [13], Paranjape (2015) [20], Gattas *et al.* (2018) [10], Krishna *et al.* (2020) [12], and Lohakare *et al.* (2021) [15] in various mango varieties are consistent with the fruit retention enhancement by CPPU treatment in the current study.

3.1.2 Fruit yield

Fruit yield was significantly and positively correlated with fruit length (r = 0.74 in location 1st and r = 0.94 in location 2nd), fruit diameter (r = 0.84 in 1st location and r = 0.95 in 2nd location), fruit weight (r = 0.96 in 1st location and r = 0.97 in 2nd location) and pulp weight (r = 0.96 in 1st location and r = 0.97 in 2nd location). CPPU treatment promotes sugar metabolism by raising levels of sucrose by increasing the transcripts of genes that regulate sucrose metabolism and upregulating the expression of cyclin genes, which are involved in cell division. This suggests that CPPU enhances cell division during the quickest growth stage, resulting in an increase in cell quantity and larger fruit size (Chen *et al.* 2022) [9]. Fruit weight increases with increasing length, diameter, or thickness (Kulkarni *et al.*, 2017) [13]. Increased fruit size and weight may be correlated to faster transit of simple sugars into fruit and involvement in cell development (Brahmachari *et al.*, 1996) [5]. The use of CPPU may benefit cell division and elongation while also accelerating protein, DNA, and RNA biosynthesis (Nickell 1986) [18].

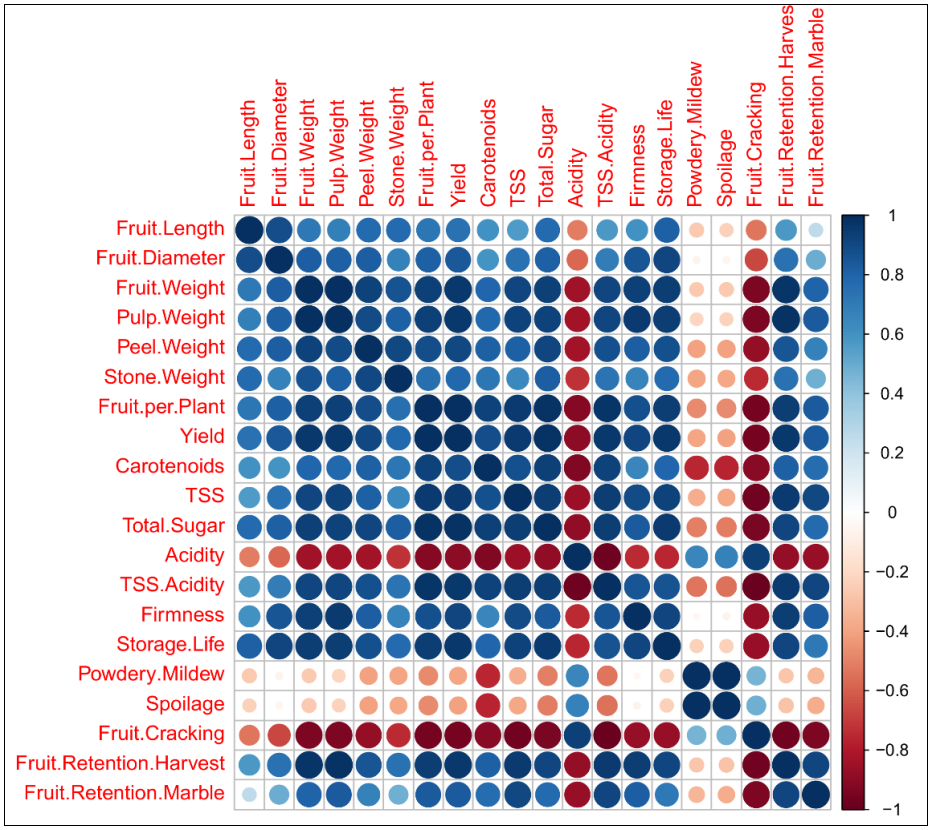

Fig 2: Correlation coefficient for location 1 (Lodhowal)

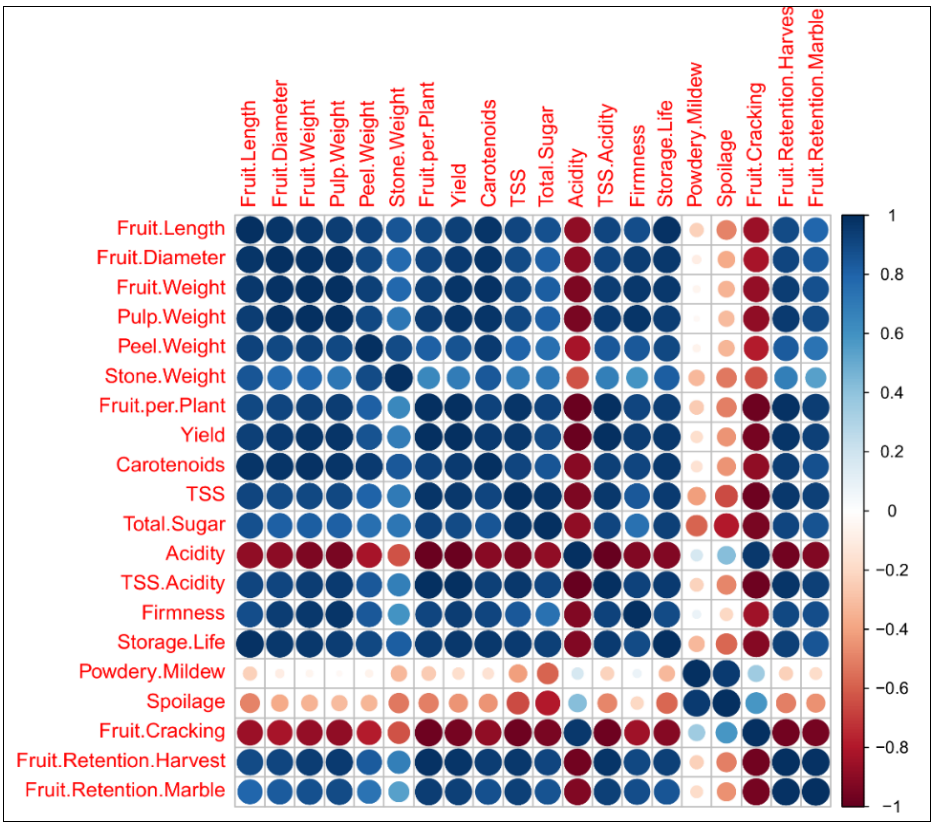

Fig 3: Correlation coefficient for location 2 (Gangian)

3.1.3 Fruit quality parameters

It is apparent from the results that the fruit yield was significantly and positively associated with fruit firmness (r = 0.91 in 1st location and r = 0.95 in 2nd location), shelf life (r = 0.96 in 1st and 2nd location), fruit TSS (r = 0.95 in 1st location and r = 0.96 in 2nd location), total sugar (r = 0.98 in 1st location and r = 0.90 in 2nd location) and TSS: acidity ratio (r = 0.96 in 1st location and r = 0.99 in 2nd location). However, fruit yield was significantly and negatively associated with fruit acidity (r = -0.89 in location 1 and r = -0.98 in location 2) and fruit cracking (r = -0.95 in locations 1 and 2). Improvement in fruit firmness and shelf life in this study may be correlated to increased

nutrient accumulation and overall improvements in fruit quality may have led to increased cell wall rigidity and decreased activity of pectin, pectin methyl esterase, and poly-galactouronase, which break down the cell wall (Valero *et al.* 2002) [26]. Mango fruit quality is mostly determined by the total amount of sugars and acids found in the pulp which were significantly enhanced in CPPU treatments. A plausible explanation for the rise in total sugar could be that CPPU activates the invertase enzyme, which converts lipids to glucose and fructose (Notodimedjo 2000) [19]. This increases water absorption, promotes leaf growth, and facilitates the transfer of food reserves to the growing sink formed by ripening fruits. When CPPU and SA were applied topically, fruit cracking at different stages of growth was greatly decreased. Fruit cracking is typically linked to the various rates at which the fruit and its peel expand. An increase in cell counts close to the fruit epidermis is associated with a decrease in fruit cracking caused by CPPU (Sano *et al.* 2018) [24].

3.2 Principal Component Analysis (PCA)

PCA biplot analysis for location 1 (Ladhowal) from the average values of two seasons (2019-20 and 2020-21) is furnished in figure 4. The data revealed that, the first 2 principal components (PC1 & PC2) explained 90.4 per cent of the total variation in location 1 (Ladhowal). Among the studied parameters, fruit retention @ marble stage and @ harvest stage, fruit diameter, fruit length, number of fruits per tree, fruit weight, pulp weight, firmness, shelf life, TSS, total sugar and TSS: acid ratios were positively associated with fruit yield. Further, PCA biplot revealed maximum values for same parameters in T6 (CPPU @ 10 ppm). However, T8-control recorded lowest values for the same parameters.

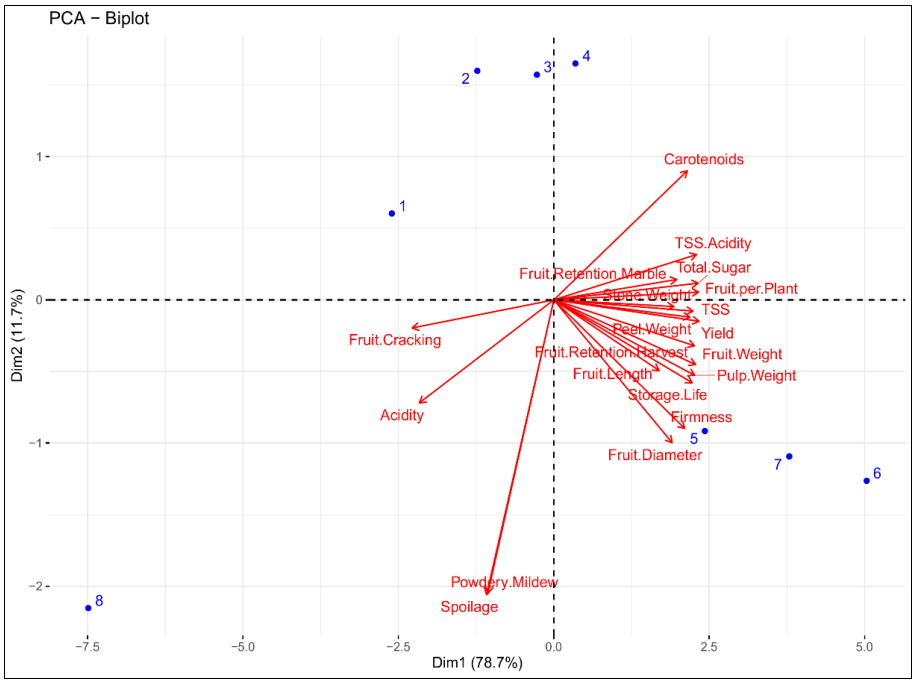

Fig 4: Principal Component Analysis for location 1 (Lodhowal)

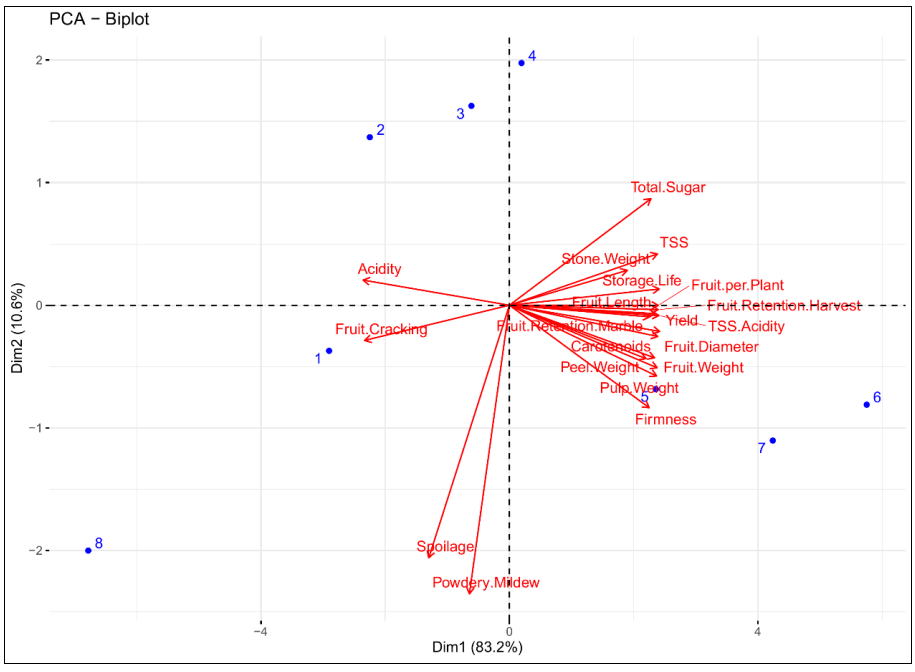

Fig 5: Principal Component Analysis for location 2 (Gangian)

PCA biplot analysis for location 2 (Gangian) from the average values of two seasons (2019-20 and 2020-21) is furnished in figure 5. The data revealed that, the first two principal components (PC1 and PC2) explained 93.8 per cent of the total variation in location 2 (Gangian). Among the studied parameters, fruit retention @ marble stage and @ harvest stage, fruit weight, number of fruits per tree, fruit diameter, fruit length, pulp weight, firmness, shelf life, TSS, total sugar and TSS: acid ratios were positively associated with fruit yield. Further, PCA biplot showed higher values for same parameters in T6 (CPPU @ 10 ppm). However, T8-control recorded lowest values for the same parameters.

4. Conclusion

The PCA and correlation investigation indicated that foliar spraying of CPPU @ 10 ppm during pea stage of fruit development considerably improved fruit retention, fruit size, and fruit yield as well as fruit quality parameters in mango cv. Dusehri.

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6. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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8. Author contribution statement

NKT: Conceptualization, Investigation, Formal analysis, Data curation, Writing - original draft, Visualization. HS: Conceptualization, Methodology, Validation, Writing - review & editing, Project Administration. ID: Resources, field supervision.

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