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IMPACT OF GAMMA IRRADIATED POLLEN ON SEXUAL COMPATIBILITY, SEED SETTING, AND FRUIT ATTRIBUTES IN GUAVA (*PSIDIUM GUAJAVA* L.) CULTIVARS

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Abstract

Pollen irradiation technique has emerged as a novel tool to induce parthenogenesis. Round shaped guava cultivars including white flesh 'Gola', pink flesh 'Surkha' and pyriform shaped cultivar white flesh 'Surahi' were self-pollinated by ^{60}Co gamma irradiated pollen at variable gamma irradiation doses viz. 100, 200, 350, 500, 700 and 900 Gy to observe metaxenial impact and induce parthenogenesis. Fruit setting and harvesting were reduced to 10% and 8% at 900 Gy of gamma irradiation, respectively. Seed setting was also reduced to half in crosses of all cultivars with irradiated pollen (102-144 seeds/fruit) compared with non-irradiated control pollen (220-250 seeds/fruit). Seeds produced in irradiated crosses were 45% lighter compared with seeds of control fruit. Round cultivars 'Gola' and 'Surkha' showed better self-compatibility compared with pyriform cultivar 'Surahi'. Pollination by irradiated pollen at higher doses improved average fruit length (12%), FL: FD (15%) and TSS: TA (50%) whereas, it decreased fruit weight (30%), TSS (20%), TA (60%), total sugars (63%) and ascorbic acid (63%) compared with fruit attributes of control fruit. Number of seeds and seed weight were positively correlated with total sugars and ascorbic acid whereas fruit weight was positively correlated with seed cavity weight and TSS. Principle component analysis (PCA) revealed greater variation in fruit biochemical traits compared with physical traits. In cluster analysis of chemical traits, higher doses of gamma irradiation were grouped separately compared with control and other treatments indicating their greater contribution to enhance variability. The irradiated pollen reduced fruit and seed setting and also influenced most of the physico-chemical traits indicating the presence of a robust metaxenial effect. The developed mutant plant populations were characterized for morphogenetic diversity. Mutation breeding could further contribute towards haploidization using induced parthenogenesis and to enhance seedlessness.

Key words Physical mutagens, Parthenogenesis, Quality traits, Incompatibility

Introduction

Guava (*Psidium guajav*a L.) is a tree which belongs to *Myrtaceae* family (Pommer & Murakami, 2009). It is grown commercially in various tropical and subtropical countries around the world for its fruits and has tremendous economic potential (Kosky *et al*., 2005; Vitti *et al*., 2020). Pakistan is the second largest guava producer in the world after India whereas other main guava producers include Mexico and Brazil (Usman *et al*., 2020). Fruit industry is dependent upon a limited number of genotypes with known quality attributes and needs genetic diversification particularly for fruit quality traits, low seediness or soft seeds which are important for consumer acceptance. Fruit trees such as guava and other fruit trees are accompanied by high heterozygosity, large canopy size and self-incompatibility (Germanà & Chiancone, 2001).

Irradiated pollen technique can be used to induce parthenogenesis, which is one of the best approaches to produce haploids due to their simple application, reproducibility, better ability of penetration, high mutation frequency and fewer disposal problems (Chahal & Gosal, 2002). This technique has been successfully used in apple, pear, loquat and different nut crops to induce haploid plants (Niazian & Shariatpanahi, 2020). The first haploid was produced in apple cultivar "Erovan" using gamma irradiated pollen at a dose of 500 to 1000 Gy (Zhang *et al*., 1988). Four genotypes of apple (*Malus domestica* L.) including Golden Delicious, R1-49, Erovan and X6677 were pollinated by gamma irradiated pollen with varying doses from 125 to 1000 Gy. The irradiated pollen influenced the fruit set, number of seeds, induced the parthenocarpic fruit and embryos. All the four genotypes produced haploid plants from immature embryos which were cultured *in-vitro* (Zhang & Lespinasse, 1991). Similarly, in loquat (*Eriobotrya japonica* L.) cultivar 'Algerie' was pollinated by gamma irradiated pollen of 'Cox', 'Changhong-3'and 'Saval Brasil' cultivars with doses ranging from 150 and 300 Gy. Haploid plants were successfully produced through parthenogenesis induced by irradiated pollen in loquat (Blasco *et al*., 2016). Likewise, in *Citrus grandis* haploid plants were produced through parthenogenesis when pollinated by gamma irradiated pollen of *C. sinensis and C. limetta* at variable doses ranging from 50 to 400 Gy. These results suggested that pollination by gamma irradiation with doses of 300-400 Gy could stimulate the process of parthenogenesis to induce haploids (Kundu *et al*., 2017). Numerous haploid plants developed using induced parthenogenesis have been reported in fruit species like grapes (Ji *et al*., 2013), citrus (Akgo *et al*., 2017; Yahata *et al*., 2017; Kawano *et al*., 2021), walnut (Grough *et al*., 2011), cape gooseberry (Garcia-Arias *et al*., 2018), nut crops (Vahdati *et al*., 2021), Kiwifruit (Musial & Przywara, 1998) and Almond (Martínez-Gómez & Gradziel, 2003).

In citrus irradiated pollen not only influence the seed formation but also help to lower the acidity content, induce early ripening, and maintain other fruit quality traits (Bermejo *et al.*, 2011). Recently it was demonstrated that use of gamma irradiated pollen in citrus induced seedlessness and reduced fruit size (Kundu & Dubey, 2017). While 'Majia' pomelo cultivar was pollinated by sour pomelo pollen irradiated with cobalt-60 gamma rays at the dose of 1000 Gy and seedless fruits were obtained. However, no significant differences of irradiated pollen were noted for fruit traits like fruit diameter, peel thickness, fruit weight, TSS, sugar content, titratable acidity and vitamin C (Yang *et al*., 2020).

Metaxenial impact of the pollen parent on fruit size and quality related attributes in guava cultivars has been previously demonstrated (Usman *et al*., 2013). However, there is no report concerning the impact of gamma irradiated pollen on fruits quality attributes in guava. Hence, the aim of the current breeding study was to evaluate the effect of gamma irradiated pollen on self-compatibility and fruit physical and quality related traits in distinct indigenous guava genotypes for induced parthenogenesis.

Materials and Methods

Plant selection and self-pollination of guava cultivars by gamma irradiated pollen: Sexually propagated, 4-5 years old fruit bearing plants of white flesh round shaped guava cultivar (Gola, G), pink flesh round shaped cultivar (Surkha, Sr) and white flesh pyriform cultivar (Surahi, SU) were selected for inbreeding using gamma irradiated pollen. Plants were grown under standard cultural practices at 15' x 15' (R x R and P x P) planting distance in the experimental fruit garden, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, (UAF), Pakistan (31.4278° N and 73.0758°E). Mature flower buds containing anthers with mature pollen grains were collected from these plants. Half of the anthers were irradiated from NIAB (Nuclear Institute for Agriculture and Biology), Faisalabad using random doses of ^{60}Co gamma irradiation i.e., (100, 200, 350, 500, 700 and 900 Gy) as previously described for Citrus (Froelicher *et al*., 2007) and loquat (Blasco *et al*., 2016) whereas the other half of the anthers were not-irradiated and used as control. Both irradiated and non-irradiated (control) anthers were allowed to dehisce at room temperature (24-25°C) for 24-36 hrs. The released pollen grains were stored at 4°C in a refrigerator. The pollen collected from the buds were tested for viability on a solid medium containing 10% sucrose, 0.1% boric acid and 1% agar within a week of storage (Yang *et al*., 2020). After viability test, the dehisced pollen grains collected from male parent plants were used to pollinate the ready to open flower buds in the female parent plants of guava cultivars (Usman *et al*., 2013). Fifty flowers were pollinated in each treatment. Pollination was carried out in the morning (8-10 AM) and afternoon (3-4 PM) during winter season. After pollination, the crossed flowers were covered with paper bags to avoid foreign pollen contamination and the bags were labelled properly. Data were collected for fruit setting (%) after two weeks of pollination and fruit harvesting (%) at crop maturity. The self-pollinated fruits of guava cultivars using non-irradiated (control) and irradiated pollen (treated) were harvested for characterization and seeds were extracted.

Physical traits: Ten mature fruit samples harvested from treated (irradiated) and untreated (control) branches were evaluated for fruit physical properties i.e., number of seeds/fruit 'NS', seed weight 'SW' (g), fruit size (fruit length 'FL' and diameter 'FD' in cm), FL: FD, fruit weight 'FW' (g), pulp weight 'PW' (g), seed core weight 'SCW' (g) and PW: SCW following UPOV (1987) descriptors. Average of ten fruits were calculated and the data were evaluated statistically.

Biochemical traits: To study the biochemical traits, all the fruits of each replication were peeled off with the help of stainless-steel knife. Small, chopped pieces of fruits (20 g) were mixed in 40 mL distilled water and blended in the juicer machine to extract the juice from each treated and untreated (control) sample of guava fruit. Following the given standard protocols, the biochemical traits were measured.

TSS (°Brix), TA (%) and TSS: TA ratio: After juice extraction, one drop was put on the refractometer prism and the total soluble solids contents of juice were determined by using digital refractometer (RX 5000, ATAGO, Japan), which expressed the TSS as °Brix. Titratable acidity (TA) was determined by using standard titration method against NaOH (Hortwitz, 1960) while TSS values were divided by TA values to calculate TSS: TA ratio.

Sugars (%) and Ascorbic Acid (mg 100 g^{-1}): Total soluble sugars (TS) and reducing sugars (RS) of the fruit juice were measured following Sadasivam & Manickam (1992). While non-reducing sugars (NRS) were measured using following equation:

$$\text{Non reducing sugar (NRS)} = \text{Total soluble sugar (TS)} - \text{Reducing sugar (RS)}$$

Five mL of the filtered aliquot was prepared by diluting 10 ml of juice with 0.4 % oxalic acid solution. The developed solution was titrated against 2, 6-dichlorophenol indophenol dye (blue color) and light pink color (appearance) was taken as end point of titration. Ascorbic acid contents were expressed as mg 100 g^{-1} fruit weight by following Ruck (1961).

Experimental design and statistical analysis: The plants were planted under completely randomized block design (CRBD) with five replications in each experiment. The fruit characterization experiment was conducted according to Completely Randomized Design (CRD) and data were analyzed using software Statistix 8.1. The significance difference among treatment means were compared using Duncan's Multiple Range (DMR) test (Li *et al*., 2008). Inter-relationship and variation among the physical and biochemical traits were assessed by Pearson's correlation coefficient (r) and principal component analysis (PCA) respectively.

Results

Fruit setting and harvesting (%): Fruit setting was higher in crosses of Round cultivars 'G' and 'Sr' compared with Pyriform cultivar 'SU' cultivar when pollinated with non-irradiated pollen (Table 1). Reduction in fruit setting (%) was more in G and Sr round cultivars when pollinated with irradiated pollen compared to SU pyriform cultivar. Overall, fruit setting (%) was reduced upto 10% at higher doses of gamma irradiation compared to control whereas fruit setting ratio was higher in crosses of round cultivars (G x G and Sr x Sr). No significant genotypic differences were observed for fruit harvesting (%) in crosses with non-irradiated and gamma irradiated pollen grains. Overall, fruit harvesting (%) was reduced at higher gamma irradiation dose 900 Gy compared to the harvest with the untreated control pollen grains (Table 1). It was concluded that fruit setting and harvesting (%) of all cultivars decreased with increase in irradiation level and self-compatibility was greater in control compared to selfing of parents with the irradiated pollen. White and pink flesh round cultivars 'G' and 'Sr' had more self-compatibility compared to white flesh pyriform cultivar 'SU'.

Determination of fruit physical traits affected by gamma irradiated pollen

Number of seeds/fruit 'NS' and Seed weight 'SW': No genotypic variability was noticed regarding number of seeds in selfed guava cultivars at control (Fig. 1). Seed setting was reduced to half in all crosses of three cultivars when pollinated with irradiated pollen grains of same variety compared with non-irradiated pollen. Seed weight of the SU x SU and Sr x Sr crosses was markedly higher compared to G x G crosses when pollinated with non-irradiated pollen. Seed weight was further reduced in the varieties when pollinated with irradiated pollen. Overall maximum seed weight was found in Sr x Sr crosses whereas seed weight was minimum in G x G crosses. Similar trends were noticed for number of seeds per gram in guava cultivars. Maximum number of seeds and seed weight were detected in control whereas minimum seeds and seed weight were found at the dose of 700 Gy (Fig. 2). It showed that irradiation treatments reduced seed setting, number of seeds and seed weight. Moreover, self-compatibility also decreased with increase in the doses of radiation. Self-compatibility was higher in round cultivars compared with pyriform cultivar.

Fruit dimensions and FL: FD: Significant increase in fruit length (FL) was observed in Sr x Sr crosses at 500 Gy and 700 Gy compared with control. Maxim fruit length and fruit diameter 'FD' was noted at the doses of 700 Gy and 100 Gy, respectively. Among genotypes, FL and FD were higher in Sr x Sr and SU x SU crosses compared with G x G cross. FL: FD was also higher at 700 Gy while lower FL: FD was noticed at 100 Gy (Fig. 2). These results indicated that the fruit dimensions and FL: FD were enhanced in selfing by increasing the dose of gamma rays. In addition, fruit size was greater in intravarietal crosses of cultivars 'Sr' and 'SU' compared with cultivar 'G'.

Fruit weight (FW), pulp weight (PW), seed core weight (SCW) and PW: SCW: Maximum fruit weight, pulp weight and seed core weight were noted in control while lowest values for these parameters were found at the dose 100 Gy, 900 Gy and 700 Gy of gamma rays, respectively (Fig. 2). Overall, FW, PW and SCW were higher in cultivar "Gola" compared with other cultivars. This investigation determined a gradual reduction of FW, PW and SCW by irradiated pollination at different doses.

Table 1. Fruit setting (%) and harvesting (%) in guava cultivars self-pollinated with gamma irradiated pollen for induced parthenogenesis.

Treatments	Crosses			Means
	G x G	SR x SR	SU x SU	
		Fruit setting (%)		
Control	63.33 ± 3.53ab	68.00 ± 3.46a	45.33 ± 4.06c-g	58.89 ± 3.92A
100	47.33 ± 4.37c-g	45.33 ± 4.81c-g	40.67 ± 4.37efg	44.44 ± 2.47BC
200	46.67 ± 4.06c-g	45.67 ± 0.88c-g	39.33 ± 2.40fg	43.89 ± 1.80BC
350	42.67 ± 3.53efg	36.67 ± 2.40g	42.67 ± 5.21efg	40.67 ± 2.19C
500	55.33 ± 5.21bcd	40.00 ± 4.16efg	45.33 ± 4.63c-g	46.89 ± 3.25BC
700	51.33 ± 3.53cde	47.33 ± 3.71c-g	49.33 ± 5.21c-f	49.33 ± 2.19B
900	44.67 ± 4.81c-g	56.00 ± 4.16bc	44.00 ± 3.61d-g	48.22 ± 2.87B
Means	50.19 ± 1.99A	48.43 ± 2.46A	43.81 ± 1.53B	
		Fruit harvesting (%)		
Control	14.00 ± 1.15	8.00 ± 1.15	16.67 ± 1.76	12.89 ± 1.46A
100	11.33 ± 1.76	11.33 ± 1.33	10.67 ± 1.76	11.11 ± 0.82AB
200	9.33 ± 2.40	10.00 ± 1.53	7.33 ± 0.88	8.89 ± 0.95BC
350	7.33 ± 0.67	10.00 ± 2.31	9.33 ± 1.20	8.89 ± 0.87BC
500	9.33 ± 1.76	8.00 ± 1.15	8.67 ± 1.33	8.67 ± 0.75BC
700	11.33 ± 3.53	8.67 ± 2.40	8.67 ± 1.76	9.56 ± 1.41BC
900	10.67 ± 1.76	6.67 ± 0.67	6.67 ± 0.67	8.00 ± 0.88C
Means	10.48 ± 0.78A	8.95 ± 0.61A	9.71 ± 0.82A	

Means sharing similar letter in a row or in a column are statistically non-significant (p>0.05)
Small letters represent comparison among interaction means and capital letters are used for overall mean

Fig. 1. Morphological variability in the fruits of guava cultivars Gola (a) and Surahi (b) self-pollinated with gamma irradiated pollen at different doses (100 Gy - 700 Gy).

Determination of fruit biochemical traits affected by gamma irradiated pollen

TSS, TA and TSS: TA ratio: No genotypic differences were noticed for TSS, TA and TSS: TA at control. Maximum TSS and TA were found at control while minimum values of TSS and TA were noted at 900 Gy. However, the ratio of TSS: TA (6.03) was enhanced at 900 Gy and the lowest ratio was observed in control (Fig. 3). These findings revealed that TSS and TA were less affected traits by gamma irradiation whereas TSS: TA ratio was massively increased by irradiation. Overall, TSS and TA were higher in round cultivars ‘G’ and ‘Sr’ while TSS: TA was greater in cultivars ‘G’ and ‘SU’.

Total Sugars (TS) and Ascorbic Acid (AA): No genotypic differences were noticed in different crosses regarding TS, reducing sugars (RS) and non-reducing sugars (NRS) when pollinated with non-irradiated pollen. With the increase in the level of gamma irradiation TS, RS and NRS were decreased in all the three crosses and the lowest level of TS was observed at the highest dose of gamma irradiation (900 Gy). Overall TS were greater in round cultivars ‘G’ and ‘Sr’ crosses compared with pyriform cultivar ‘SU’. The highest values of TS, RS, and NRS were noticed at control while the lowest values of these traits were found at 900 Gy (Fig. 3). Ascorbic acid contents were greater in SR x SR crosses under control conditions compared with G x G and SU x SU crosses. Like sugars, AA contents were also reduced with the increase in irradiation level and the lowest values of AA were found at 900 Gy. Overall, AA contents were maximum in Sr x Sr and minimum in G x G crosses. Conclusively, like other biochemical traits, sugars and ascorbic acid were also decreased with the increase of irradiation level of the pollen grains.

Correlation analysis of physical and biochemical traits: Physical and biochemical traits of fruit in guava cultivars revealed significant correlation. Fruit setting (%) and harvesting (%) were highly positively correlated with NS and RS. NS were highly positively related with TS, RS and AA. SW was directly related with TSS, TA, TS, NRS and AA. Fruit and pulp weight were strongly related with SCW and TSS. Similarly, TSS and TS were highly positively related with TSS: TA, RS, NRS and AA. TS sugars, RS and NRS had strong positive relation with ascorbic acid (Table 2).

Principal component analysis (PCA): Principal component analysis (PCA) for fruit of three different cultivars, self-pollinated using gamma irradiated of different levels, pollen grains showed great variation for both fruit physical (Fig. 4a, 5a) and quality attributes (Fig. 4b, 5b).

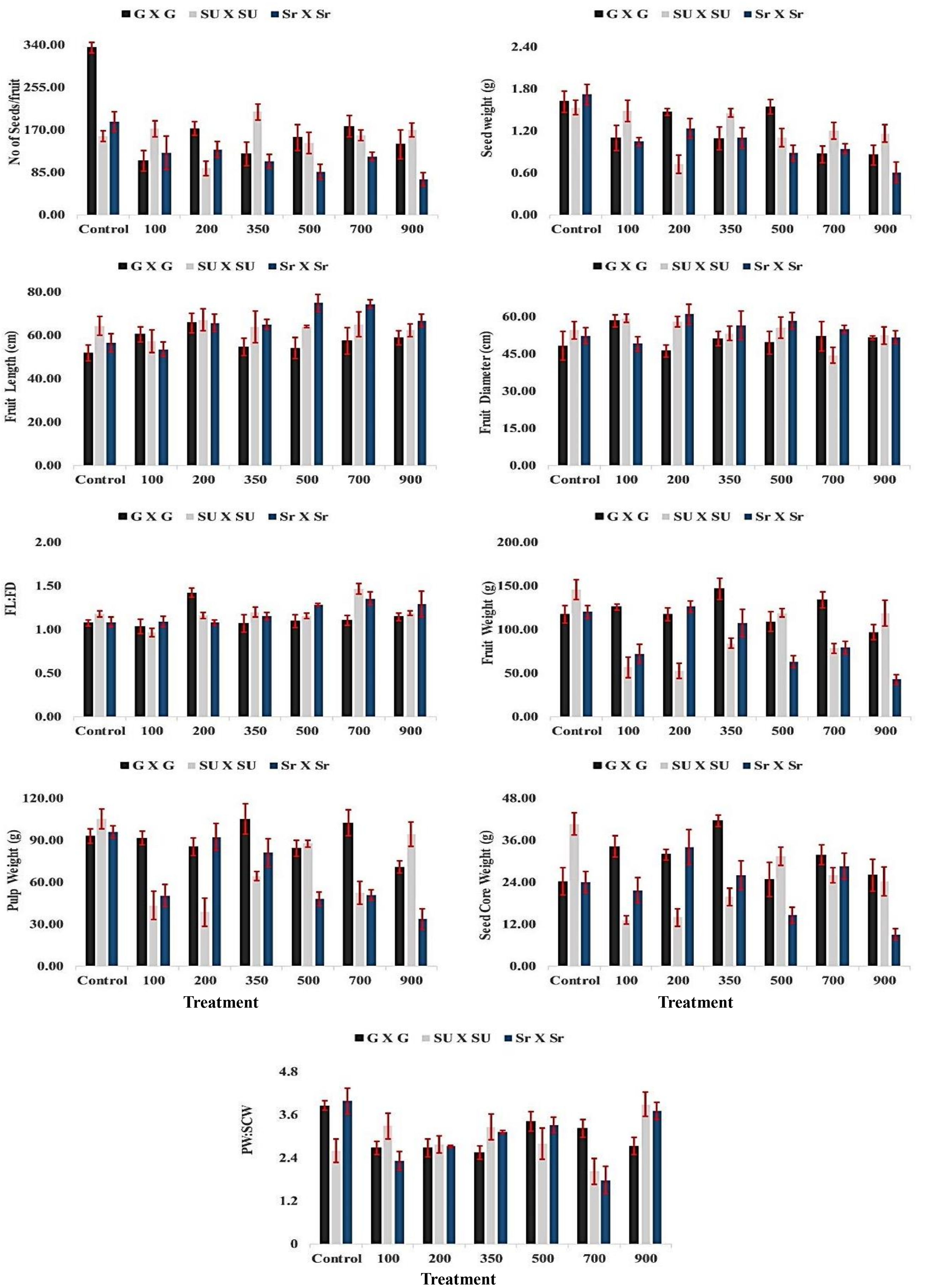

Fig. 2. Physical traits of fruit of guava cultivars self-pollinated with gamma irradiated pollen. Figures include fruit length (cm), fruit diameter (cm), FL: FD, fruit weight (g), pulp weight (g) and seed core weight (g).

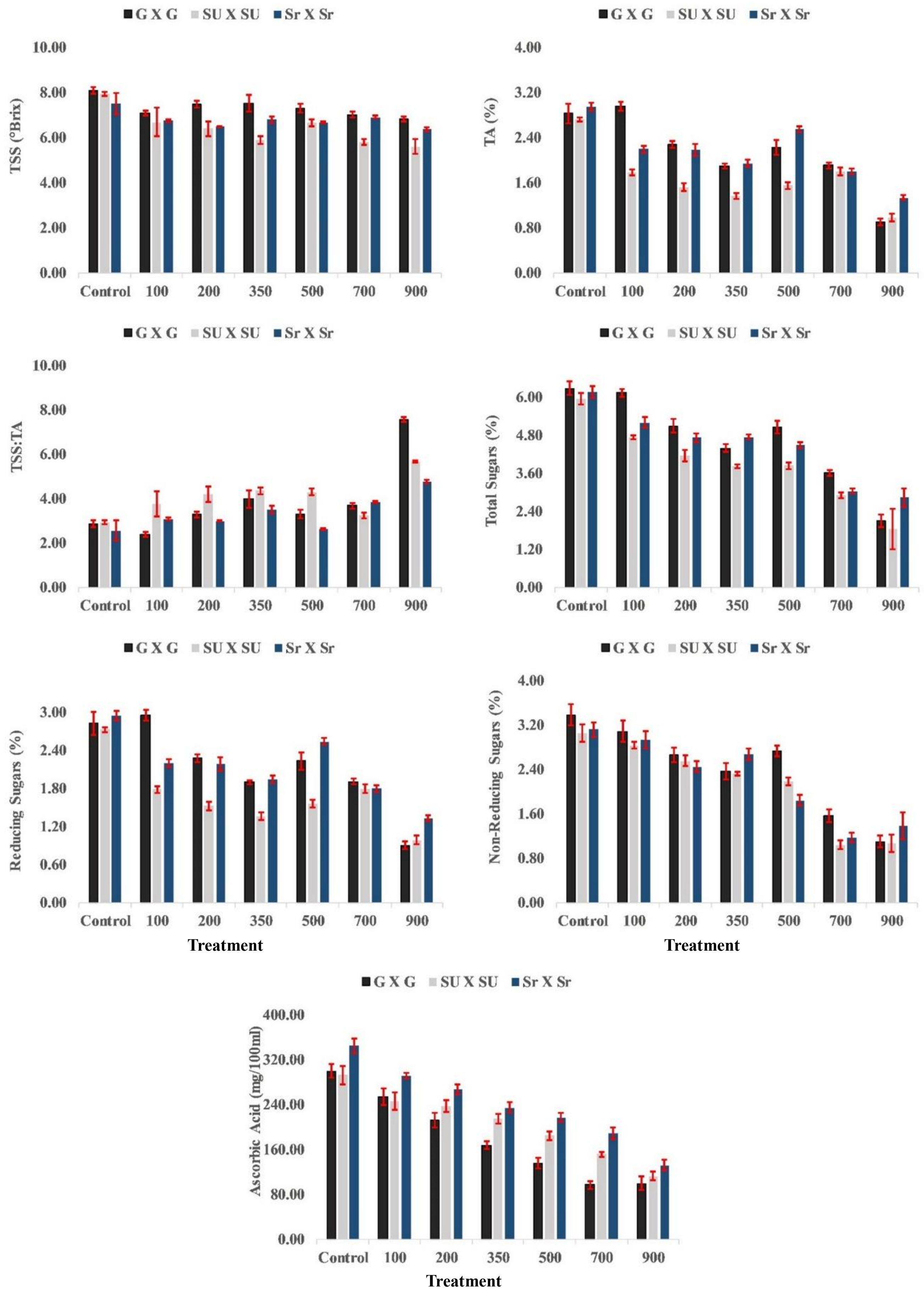

Fig. 3. Fruit quality of guava cultivars self-pollinated with gamma irradiated pollen. Figures include TSS (°Brix), Titratable Acidity (g/L), total sugars (%), reducing sugars (%), non-reducing sugars (%) and ascorbic acid contents (mg/100ml juice).

Table 2. Pearson's correlation matrix of physicochemical fruit traits of guava cultivars self -pollinated with gamma irradiated pollen.

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20
V2	0.998**																			
V3	0.763**	0.759**																		
V4	0.722**	0.717**	0.984**																	
V5	0.778**	0.782**	0.689**	0.678**																
V6	0.203	0.213	0.080	0.099	0.552**															
V7	0.623**	0.616**	0.649**	0.616**	0.473**	0.470**														
V8	-0.229	-0.223	-0.365*	-0.393*	-0.228	-0.074	-0.174													
V9	-0.230	-0.209	-0.035	-0.080	-0.110	0.116	-0.248	0.422*												
V10	-0.067	-0.074	-0.354	-0.349	-0.167	-0.175	0.003	0.702**	-0.341											
V11	0.036	0.046	-0.053	-0.056	0.228	0.313	-0.063	-0.344	-0.123	-0.275										
V12	0.082	0.092	-0.030	-0.036	0.252	0.338	-0.066	-0.392*	-0.118	-0.332	0.986**									
V13	-0.095	-0.084	-0.108	-0.104	0.130	0.199	-0.044	-0.165	-0.119	-0.084	0.894**	0.806**								
V14	0.325	0.323	0.099	0.082	0.169	0.198	-0.035	-0.271	0.030	-0.322	-0.004	0.159	-0.443*							
V15	0.116	0.111	-0.051	-0.030	0.220	0.492**	-0.263	-0.335	-0.145	-0.261	0.562**	0.581**	0.432*	0.175						
V16	0.054	0.059	-0.163	-0.127	0.127	0.469**	-0.344	-0.169	-0.032	-0.162	0.394*	0.428*	0.247	0.281	0.755**					
V17	0.117	0.110	0.302	0.259	0.087	-0.320	0.421*	0.073	-0.002	0.078	-0.209	-0.230	-0.122	-0.213	0.499**	0.896**				
V18	0.423*	0.428*	0.319	0.334	0.577**	0.504**	0.077	-0.290	0.008	-0.315	0.304	0.334	0.178	0.234	0.631**	0.737**	0.571**			
V19	0.700**	0.705**	0.733**	0.729**	0.743**	0.314	0.460*	-0.217	-0.001	-0.231	0.168	0.176	0.120	0.077	0.333	0.388*	-0.218	0.770**		
V20	0.091	0.095	-0.101	-0.076	0.285	0.505**	-0.239	-0.267	0.005	-0.290	0.323	0.366*	0.161	0.312	0.650**	0.768**	0.656**	0.859**	0.339	
V21	0.316	0.327	0.395*	0.433*	0.578**	0.585**	-0.016	-0.248	0.117	-0.353	0.129	0.151	0.052	0.143	0.395*	0.596**	0.495**	0.780**	0.636**	0.651**

* = Significant (p<0.05); ** = Highly significant (p<0.01)

V1 = Total fruit setting , V2 = Fruit setting (%) , V3 = Total fruit harvest, V4 = Harvesting (%) , V5 = No of seeds/fruit , V6 = Seed weight , V7 = No of seeds/g , V8 = Fruit length , V9 = Fruit diameter V10 = FL: FD , V11 = Fruit weight , V12 = Pulp weight , V13 = Seed core weight , V14 = PW: SCW, V15 = TSS (Total soluble solids) , V16 = TA (Titratable Acidity), V17 = TSS: TA, V18 = TS (Total sugars), V19 = RS (Reducing sugars), V20 = NRS (Non-reducing sugars), V21 = Ascorbic Acid

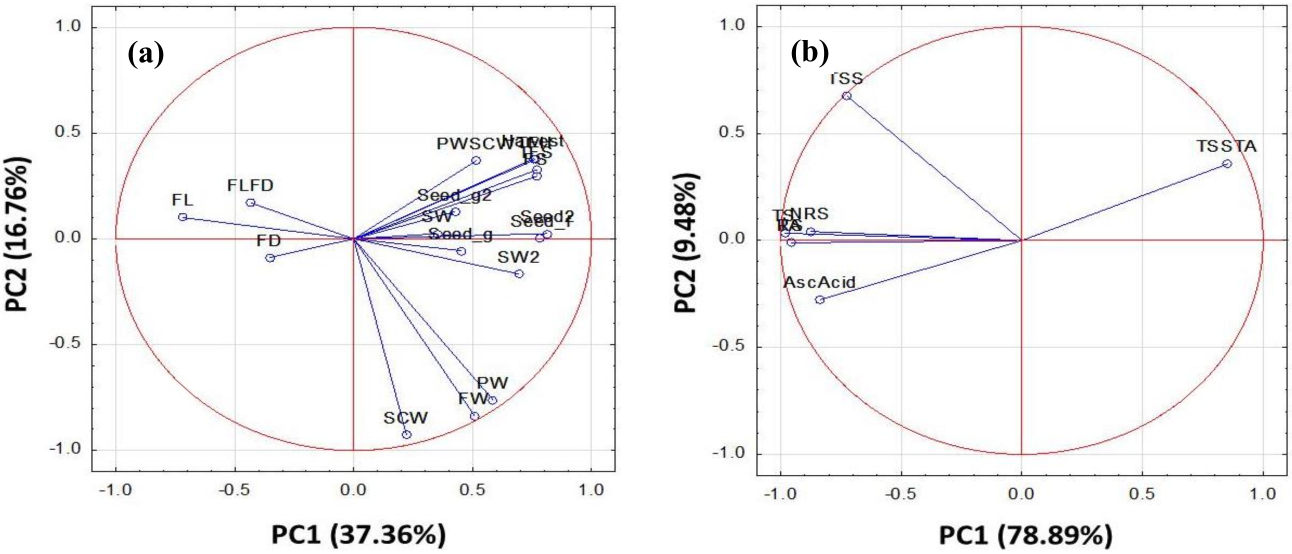

Fig. 4. Projection of the fruit physical and biochemical variables of the (PCA) affected by mutation breeding.

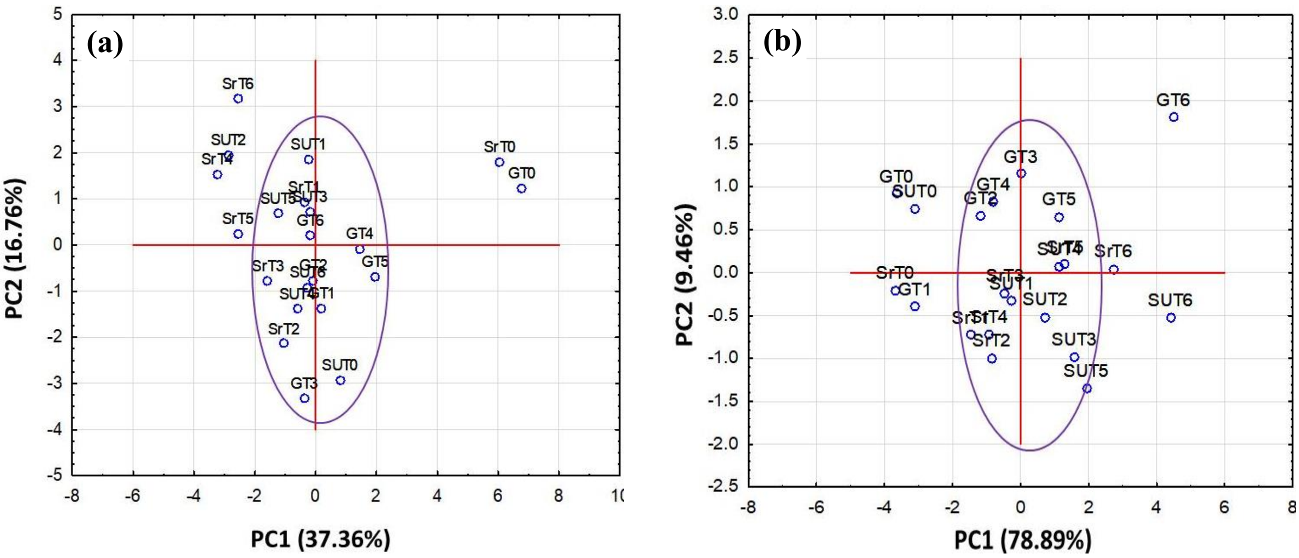

Fig. 5. Projection of the varietal and radiational variables of the (PCA) affected by gamma irradiation in self-pollinated guava cultivars.

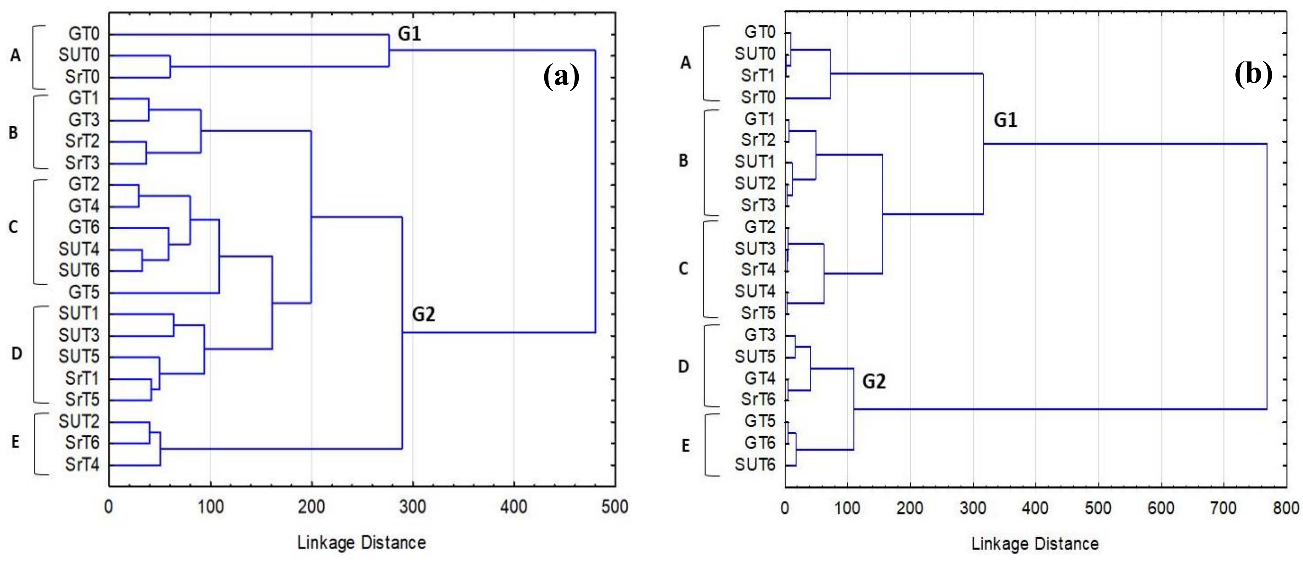

Fig. 6. Similarity dendrograms obtained from fruit physical (a) and biochemical (b) attributes in guava cultivars self-pollinated by gamma irradiated pollen.

The first two components explained (54.12%) the cumulative variance, with PC1 and PC2 accounting for (37.36%) and (16.76%) in physical traits. PC1 was negatively correlated to fruit size (FL, FD and FL: FD) and positively correlated to rest of the physical attributes. Whereas PC2 was positively correlated with FL, FL: FD, NS and PW: SCW however, negatively correlated with seed and fruit weight (SW, FW, PW and SCW) (Fig. 4a). In quality factor plane, the first two components of PCA described (88.35%) the cumulative variance, with PC1 and PC2 accounting for (78.89%) and (9.46%), respectively. PC1 was positively corelated with TSS: TA but had negative correlation with sugars (TS, RS, NRS) and AA. PC2 was positively correlated with sugars (TS, RS, NRS) and TSS: TA while it was negatively correlated with AA (Fig. 4b).

Likewise in the second PCA plot, based on 1st and 2nd components (37.36%) and 16.76%) of the fruit physical traits showed less variation (Fig. 5a) compared with the 1st and 2nd components (78.89% and 9.46%) of the fruit quality traits (Fig. 5b). In both physical and quality attributes, all the three cultivars pollinated with non-irradiated pollen (T_0) and pollen irradiated at 900 Gy (T_6) were clustered separately as outliers, while rest of the cultivars and treatments were clustered closely in both cases (Fig. 5a, b). The analysis of PCA provided a more in-depth approach to clarify the effect of irradiation on fruit physical and quality attributes in guava cultivars.

Hierarchical clustering analysis (HCA): Hierarchical clustering analysis identified two main groups (G1, G2) with five sub-groups (A-E) of guava cultivars affected by different doses of gamma irradiation through assessment of physical (Fig. 6a) and quality traits (Fig. 6b). In physical traits, the control crosses were clustered under group G1 whereas, the rest of the crosses with irradiated pollen grains were clustered as group G2. The subgroups B-C mainly comprised of cluster of various treatments in cv. 'G', C-D subgroups comprised of treatments of cv. 'SU' and subgroups D-E showed cluster of treatments of cv. 'Sr'. In quality traits, the crosses pollinated by pollen irradiated at higher doses (T_4-T_6) were grouped under G2 while the rest of the treatments (T_0-T_3) were grouped under G1. These dendrograms showed less variation within sub-groups and greater variations between different sub-groups. Linkage distance represented the degree of association between the group members, with larger values indicating that association was more significant.

Discussion

Pollen irradiation technique has been extensively used in different crops to induce parthenogenesis (Lacadena, 1974; Raquin *et al.*, 1989). Higher doses of gamma rays precisely influenced the viability of the pollen (Froelicher *et al.*, 2007) and subsequently affected fruit setting (Lotfi *et al.*, 2003), fruit harvesting and seed set (Kurtar, 2009; Kundu & Dubey, 2017). In the present study, fruit setting (%), fruit harvesting (%) and seed setting were greatly affected by irradiated pollen in all guava cultivars during self-pollination. Fruit setting was higher in round shaped Gola cultivars 'G' and 'Sr' compared with pyriform Surahi cultivar 'SU' indicating genotypic variability for fruit setting and greater self-compatibility in round cultivars compared with pyriform cultivar. However, no significant genotypic variability was noticed in fruit harvesting. Among irradiation treatments, both fruit setting and fruit harvesting were reduced (10% and 4%, respectively) in crosses pollinated with irradiated pollen compared with crosses pollinated with non-irradiated pollen (control) indicating a strong effect of gamma irradiation on pollen viability, pollination and fertilization frequency. Interestingly, seed setting was also reduced to half (104-129 seeds) in all the crosses of three cultivars when pollinated with the irradiated pollen compared with non-irradiated control pollen (221-251 seeds). The considerable reduction in fruit and seed setting could be attributed to reduced pollen viability caused by higher gamma irradiation. Similar findings were reported in other fruit crops like sweet cherry (Höfer & Grafe, 2003), apple (Zhang & Lespinasse, 1991), pear (Bouvier *et al.*, 1993), citrus (Froelicher *et al.*, 2007), plum (Peixe *et al.*, 2000) and melon (Lotfi *et al.*, 2003). Seed weight was also reduced up to 45% when pollinated with the irradiated pollen compared with control. Irradiation dose, time and genotype precisely affects and reduce the seed set and develop lighter seeds. In the current study, higher dose of gamma irradiation reduced fruit-set and developed guava fruit had less seeds (parthenocarpic) with reduced seed weight. These findings support the tendency of parthenogenesis (haploid production) in guava, which had agreement with prior studies in other crops (Kurtar, 2003; De Menezes *et al.*, 2005; Blasco *et al.*, 2016; Yang *et al.*, 2020). In addition, self-compatibility was higher in white and pink flesh round cultivars 'Gola' and 'Surkha' compared to white flesh pyriform cultivar 'Surahi'.

The effect of irradiated pollen on physico-chemical traits of self-pollinated fruits of guava cultivars were also investigated. Increase in fruit length (FL) was noted up to 10-12% in Sr x Sr crosses at higher doses of gamma irradiation compared with control. The fruit dimensions (length and diameter) and FL:FD were enhanced when selfed with pollen irradiated at higher dose of gamma rays. Similar trend of enhanced fruit dimensions was found at increased level of gamma irradiations in *C. grandis* and *C. limetta* (Goldenberg *et al.*, 2014; Kundu *et al.*, 2014). Gradual reduction in FL and FD by exposure to irradiated pollen was observed in citrus which is contrary to our findings (Kundu & Dubey, 2017). Hence, increase in fruit size by irradiated pollen demonstrated the existence of metaxenial effect in self-irradiated crosses of guava cultivars. Metaxenial effect of pollen parent for fruit size and quality has been demonstrated in intervarietal crosses of guava using non-irradiated pollen (Usman *et al.*, 2013). Meanwhile, the highest fruit weight, pulp weight, seed core weight and PW: SCW were noted in control while the lowest values for these parameters were found at higher doses of gamma rays. The fruit weight at higher dose of gamma rays was reduced up to

32% compared with control. These outcomes are consistent with the preceding results (Zamir *et al.*, 2009; Zheng *et al.*, 2009; Goldenberg *et al.*, 2014; Kundu *et al.*, 2017; El-Mageid & Al-Kfrawey, 2018). On the other hand, maximum TSS and TA were observed in control while minimum TSS and TA values were noted at the dose of 900 Gy. Ratio of TSS:TA is an important trait in fruit quality parameters. Interestingly, the ratio of TSS:TA (6.03) was remarkably enhanced at 900 Gy compared with the lowest TSS:TA (2.78) observed in control. These findings revealed that both TSS and TA values were reduced at higher doses of gamma irradiation, however, TSS:TA ratio was increased by irradiation. This is attributed to higher reduction in TA at higher gamma irradiation as reported in citrus (Bermejo *et al.*, 2011; Çimen *et al.*, 2020). The highest values of TS, RS and NRS were noticed in control while the lowest values of these traits were found at the dose of 900 Gy. These results indicated that most of the fruit biochemical traits decreased with increasing the doses of gamma irradiation. Such investigation is consistent with the results described by Yang *et al.*, (2020).

Fruit size, volume, weight, and fruit quality attributes are related to number of seeds in fruit. In guava, number of seeds were found to be strongly related to total sugars and ascorbic acid. Decrement in fruit weight, total soluble solids, total sugars and ascorbic acid with higher gamma irradiation could also be attributed to reduced seed setting. Number of seeds play a key role in enhancing fruit size, weight and fruit quality via growth hormones as demonstrated in citrus (Pandolfini, 2009). Furthermore, the poor growth response of pollen grains irradiated at higher doses may lead to pseudo-fertilization and reduced auxin production resulting in poor fruit development in weight and fruit quality (Kundu & Dubey, 2017).

Pollination using gamma irradiated pollen can promote parthenogenesis, reduce number of seeds, and produce haploids in guava by interrupting the normal process of fertilization. We demonstrate here the responses of mutation breeding to fruit size, weight, quality, seed setting and induction of parthenogenesis as first report. Moreover, morphological investigation as well as fruit characterization of these plants will be valuable for genotype diversification, genetic analysis, and further breeding implications. Selection and morphogenetic characterization of the developed mutant plant population is underway.

Conclusion

White and pink flesh round cultivars "Gola" and "Surkha" revealed greater self-compatibility compared to white flesh pyriform cultivar "Surahi". Use of gamma irradiated pollen for self-pollination in guava cultivars reduced fruit harvest, seed setting, seed weight and total sugars whereas it enhanced fruit size and ratio TSS/TA indicating existence of a metaxenial effect. Conclusively, pollination by irradiated pollen may be utilized as an effective technique to alter fruit attributes and induce parthenogenesis to develop haploids in guava.

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