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Graft-induced variations on morphological, biochemical and molecular parameters in apple (*Malus* x *domestica* Borkh.)

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Though grafting in plants is in practice since ages only limited information is available on the graft-induced morphological, biochemical and molecular variations. The mechanism(s) by which rootstocks influence scion vegetative growth and development, and *vice versa*, are not fully understood. Therefore, in the present investigation, we studied graft-induced variations in different apple stionic combinations using scion (Scarlet Gala and Red Fuji) and rootstocks (MM111, MM106, M7, M9 and M26). Under morphological variations, significant difference was found in all the parameters *viz.* leaf area, shoot length, internodal length and fruit characters except petiole length and fruit firmness. Likewise, significant difference was also observed in the subjected stionic combinations based on total phenolic contents, total free amino acids and peroxidase enzyme activity. These results were further validated using DNA based molecular markers SSR. However, no polymorphism was observed in both the stionic combinations at DNA level. These results imply that rootstocks may influence morphological and biochemical parameter of the scion during graft-union but it does not influence genetic constitution of scion at DNA level. Further studies at RNA or protein level are required to unravel the reasons behind these variations.

Keywords: Scion, Stionic combinations

Apple (*Malus* x *domestica* Borkh.) belong to Rosaceae family, popularly known as king of temperate fruits having chromosome no. x=17 and is one of the most important temperate fruit crop grown all over the world[1]. In India, about 3,08,000 ha are under apple cultivation with the yield of 7.52 MT/ha (FAO, 2019)[2]. Apple trees are generally propagated by grafting to improve productivity and control damage caused by soil-borne disease and abiotic stress[1]. The success of grafting primarily depends on the compatibility of the stock and scion to enable rapid development of vascular connections that is why the stock and scion must be closely related taxonomically (i.e. genetically) to form functional graft union[3], which, in turn, will allow quick resumption of the growth of both the root and the canopy[4].

Though grafting has been practiced since ancient period in horticulture and agriculture, only little information is known about the morphological, biochemical, molecular mechanism of rootstock-regulation of scion's phenotypes. Changes in graft anatomy are often evident when stem tissue that restricts its vegetative growth[5]. The changes in morphology of leaves, flowering and fruits due to genetic changes induce by grafting that has potential in genetic improvement were reported when potato used as scion grafted on tomato rootstock resulted in changing the leaf shape of the potato and graft-transmissible RNA from the tomato rootstock to potato scion was reported which can lead to change in leave morphology of potato scion[6].

During graft union formation, several biochemical pathways are affected such as hormones and metabolism of phenolic compounds. The hormones are important class of compounds implicated in the development of the graft union. For instance, auxins are released from vascular strands of both stock and scion to induce the differentiation of vascular tissues[7]. Some results were found in grapevines such as 'Leaves of flame seedless' and 'sharad seedless' vines grafted on Dogridge rootstock, which accumulate more ABA at 50% moisture stress, resulting in increased water-use efficiency as compared to their own rooted vines of the same cultivars[8].

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The genetic variations may occur during grafting due to transfer of genetic material from the stock to the scion across the graft junction. During DNA replication, the DNA fragments transferred from the stock plant and integrate into the chromosomes of scion cells by homologous recombination with the scion genome[9]. Later, to detect the evidence of DNA exchange between stock and scion in citrus grafts, experiments were carried out and found to be unsuccessful[10]. A few reports on change in scion gene expression upon grafting have also been reported[11,12]. To compare the effect of dwarfing and semi-vigorous rootstocks on gene expression in the scion, cDNA amplified fragment length polymorphism were used and observed the differential regulation of various transcription factors and genes involved in signaling processes[11]. When scion of genetically identical apple was grafted on rootstocks with different genotypes, several genes are differentially expressed in genetically identical apple scions[12].

To our knowledge, graft-induced variations in plants, still remains under-explored. Therefore, in the present study, we investigated graft-induced variations in different stionic combinations of apple and for understanding the mechanism of how the rootstock affects the scion.

Materials and Methods

Experimental material

The experimental plant material consisting of two apple varieties 'Scarlet Gala' & 'Red Fuji' grafted on five different apple rootstocks i.e., M9, M26, M7, MM106 and MM111 of same age (5 years old) were procured from the Regional Horticulture Research & Training Station, Mashobra, Shimla (HP) and the Department of Fruit Science, Dr Y S Parmar UHF, Nauni, Solan (HP), respectively. The morphological variations induced by grafting such as leaf area, petiole length, shoot length, internodal length, fruit size, fruit weight, fruit shape, fruit colour, fruit firmness and total soluble solids (TSS) were recorded for both the year. Biochemical characterization was carried out by total phenol contents, total amino acid contents and peroxidase enzyme activity for both the years. Molecular variation was determined by SSR markers which were further validated to validate the effect of rootstocks on the scion after grafting. The data were analyzed on the observations drawn on consecutive two years (2016-18).

Morphological characterization (based on tree and fruit characters)

The parameters such as leaf area, petiole length, internode length and shoot length were considered with respect to tree characters from mid of July to mid of August (for two years 2016-2018). Likewise, parameters, such as fruit size, fruit colour, fruit shape, firmness and TSS (Total soluble solids) were analyzed after harvesting of fruits from both the varieties grafted on different rootstocks.

Biochemical characterization

The biochemical characterization was done based on total phenolic compounds, total free amino acids and peroxidase enzyme activity. Total phenolic compounds were extracted in ethanol from the dried leaves of both the varieties grafted on different rootstocks and individual rootstocks using Folin-Ciocalteu method[13], with catechol as the standard. Similarly, total free amino acids were estimated from the leaves of both the apple varieties grafted on different rootstocks and individual rootstocks using Ninhydrin method[14], with leucine as the standard. While, the extraction and assay of POX were carried out as per the method described by Putter[15], the reaction mixture contained 3 mL 0.1 M phosphate buffer (pH 7.0), 0.05 mL 20 mM guaiacol, 0.03 mL H_2O_2 and 0.1 mL enzyme. The enzyme activity was detected by increase in the absorbance at 436 nm in a spectrophotometer.

Molecular characterization

Isolation and purification of DNA was carried out from the fresh and young leaves of rootstocks and both the varieties grafted on different rootstocks of apple using CTAB method[16]. In the present study, already reported 25 SSR markers were used for amplifying the isolated genomic DNA. Reaction conditions and optimum concentrations of various components *viz*., 25-50 ng template DNA, 1.5 mM-2.5 mM $MgCl_2$, 0.2 mM-1 mM dNTPs, 0.3U-1U Taq DNA polymerase and 15-35 pmoles primers were standardized. 15 µL of mixture for respective PCR analysis was prepared. The PCR reactions were carried in a thermo cycler (Applied Biosystem, USA) and amplified DNA fragments were further separated on 3% agarose gel stained with ethidium bromide, viewed under UV light with the help of transilluminator and photographed with the help of gel documentation system (Syngene, UK).

Statistical analysis

The data recorded was analyzed using MS-Excel and OPSTAT[17]. The mean values of data were

subjected to analysis of variance for using randomized block design (Factorial) and completely randomized design (Factorial) as described by Panse & Sukhatme[18].

Results

Morphological characterization

The significant effect of rootstock on the tree characters of scion was reported in the observations taken for two years i.e. 2016 to 2018. The characters such as leaf area, shoot length and internode length except petiole length were found to be affected due to rootstocks for both the stionic combinations. Scarlet Gala grafted on rootstock M7 and Red Fuji grafted on rootstock M26 were found to have largest leaf area (Table 1) but in case of rootstocks, the largest leaf area was found in M7 whereas, the rootstock M26 have the smallest leaf area (Table 1). Significant difference was observed in internode length among both the grafted varieties and rootstocks. The internode length of the Scarlet Gala varied from 2.32 cm to 3.19 cm and for Red Fuji, it varied from 2.32 cm to 3.08 cm when grafted on different rootstocks (Table 1). Likewise, in the rootstocks internode length varied from 1.65 cm to 2.03 cm (Table 1). The highest shoot length was observed when Scarlet Gala grafted on rootstock M26 (55.25 cm) and Red Fuji grafted on MM111 (36.38 cm) (Table 1) and in the rootstocks the highest shoot length was observed in M26 (Table 1).

Fruit characters of both the varieties were significantly influenced by different rootstocks except fruit firmness (Table 2). The largest fruit size, fruit weight was obtained when Scarlet Gala grafted on the rootstock M26 and Red Fuji grafted on the rootstock MM106 and lowest fruit weight was obtained when both the targeted varieties grafted on rootstock MM111. The most of fruits were in globose, conic and obloid shape in both the apple varieties (as per the standards of UPOV). The most of the fruit colour of the Scarlet Gala was of Red Group 47A and Red Fuji was of Greyed Purple184A (as per the colour chart of the Royal Horticultural Society, London). The maximum TSS was obtained when both the varieties grafted on rootstock MM106 and the total soluble contents were varied from 10.15˚B to 11.15˚B in Scarlet Gala and 9.05˚B to 10.10˚B in Red Fuji.

Biochemical characterization

Significant effect of the rootstocks was observed on the phenolic contents of leaves in both the varieties. The phenolic contents for Scarlet Gala varied from

Table 1 — Analysis of tree characters in different apple stionic combinations and rootstocks

Apple stionic combinations

Scion / Rootstock	Leaf area (cm^2)						Petiole length (cm)						Internodal length (cm)						Shoot length (cm)					
	Scarlet Gala			Red Fuji			Scarlet Gala			Red Fuji			Scarlet Gala			Red Fuji			Scarlet Gala			Red Fuji		
	Year 2017	Year 2018	Pooled	Year 2017	Year 2018	Pooled	Year 2017	Year 2018	Pooled	Year 2017	Year 2018	Pooled	Year 2017	Year 2018	Pooled	Year 2017	Year 2018	Pooled	Year 2017	Year 2018	Pooled	Year 2017	Year 2018	Pooled
MM111	19.12	19.20	19.16	19.25	19.29	19.27	3.23	3.18	3.20	3.28	3.70	3.49	2.51	2.45	2.48	2.32	2.28	2.29	39.75	40.25	40.00	35.75	37.00	36.38
M7	24.79	25.28	25.04	22.91	22.45	22.68	3.18	2.90	3.04	3.35	3.95	3.65	2.80	2.86	2.83	3.08	3.02	3.05	50.75	51.75	51.25	23.25	24.75	24.00
MM106	21.89	22.24	22.06	25.97	25.56	25.76	3.45	3.45	3.45	3.75	3.93	3.84	3.19	3.12	3.15	2.72	2.75	2.74	48.25	49.00	48.63	33.25	33.75	34.50
M26	20.60	19.24	19.92	26.97	26.23	26.60	3.43	3.33	3.38	3.23	3.40	3.31	2.91	2.87	2.89	2.47	2.52	2.49	54.50	56.00	55.25	29.50	31.50	30.50
M9	22.57	21.85	22.21	24.25	23.90	24.07	3.40	3.63	3.51	3.08	2.85	2.96	2.32	2.46	2.39	2.69	2.64	2.66	25.25	25.75	25.50	31.00	31.25	31.13
Mean	21.79	21.56		23.87	23.49		3.34	3.30		3.34	3.57		2.74	2.75		2.66	2.64		43.70	44.55		30.55	31.65	

Apple rootstocks

Scion / Rootstock	Leaf area (cm^2)			Petiole length (cm)			Internodal length (cm)			Shoot length (cm)		
	Year 2017	Year 2018	Pooled	Year 2017	Year 2018	Pooled	Year 2017	Year 2018	Pooled	Year 2017	Year 2018	Pooled
MM111	20.90	20.56	20.73	1.93	1.93	1.93	2.00	1.95	1.98	25.25	27.75	26.50
M7	22.12	22.47	22.30	1.84	1.60	1.71	2.03	1.96	1.99	22.00	23.25	22.63
MM106	21.81	21.10	21.45	1.50	1.58	1.54	1.65	1.66	1.65	27.75	32.00	29.88
M26	18.64	19.23	18.93	1.78	1.75	1.76	1.78	1.80	1.80	30.50	32.25	31.38
M9	20.68	20.65	20.66	1.60	1.63	1.61	1.73	1.67	1.69	23.75	25.75	24.75
Mean	20.83	20.80		1.73	1.70		1.84	1.81		25.85	28.20	

Table 2 — Analysis of fruit characters in different apple stionic combinations and rootstocks

Root stock / Scion	Fruit Length (mm)		Fruit Diameter (mm)		Fruit L/D ratio		Fruit weight (g)		Fruit Shape		Fruit Colour		Firmness (kg/cm^2)		TSS (°B)	
	Scarlet Gala	Red Fuji	Scarlet Gala	Red Fuji	Scarlet Gala	Red Fuji	Scarlet Gala	Red Fuji	Scarlet Gala	Red Fuji	Scarlet Gala	Red Fuji	Scarlet Gala	Red Fuji	Scarlet Gala	Red Fuji
MM111	47.42	49.71	57.82	60.53	0.82	0.82	80.50	86.25	Globose, Obloid	Globose	Red Group47A	Greyed Purple183D	11.68	11.40	10.90	9.70
M7	49.96	48.83	61.34	60.02	0.82	0.82	94.88	89.75	Obloid, Globose	Conic, Globose	Red Group47A	Greyed Purple184B	12.40	12.25	10.60	9.93
MM106	48.86	51.92	60.14	65.64	0.81	0.79	90.38	109.38	Conic, Globose	Globose	Red Group47A	Greyed Purple184A	11.47	11.70	11.15	10.10
M26	56.39	49.87	63.37	61.18	0.89	0.82	106.00	90.13	Conic, Globose	Globose	Red Group47B	Greyed Purple184A	12.08	10.71	10.15	9.93
M9	52.94	52.58	61.80	60.17	0.86	0.88	94.88	93.13	Globose	Obloid, Globose	Red Group46A	Greyed Purple184A	11.18	12.73	10.45	9.05

[L/D: Length and Diameter ratio; TSS: Total Soluble Sugar]

Table 3 — Total phenol, total free amino acid content and peroxidase activity in different apple stionic combinations and rootstocks

Rootstock / Scion	Apple stionic combinations															
	Total phenolic contents (mg/g DW)						Total free amino acid contents (mg/g)						Peroxidase enzyme activity (EU)					
	Scarlet Gala			Red Fuji			Scarlet Gala			Red Fuji			Scarlet Gala			Red Fuji		
	Year	Year	Pooled	Year	Year	Pooled	Year	Year	Pooled	Year	Year	Pooled	Year	Year	Pooled	Year	Year	Pooled
Rootstock	2017	2018		2017	2018		2017	2018		2017	2018		2017	2018		2017	2018	
	126.52	127.71	127.11	107.14	112.05	109.59	14.02	13.17	13.59	12.46	12.78	12.62	53.00	59.59	56.29	61.51	67.27	64.39
MM111	±3.10	±2.88		±2.65	±2.30		±0.55	±0.64		±0.44	±0.58		±1.90	±2.28		±1.31	±1.43	
	106.60	91.40	99.00	116.83	119.96	118.39	11.17	11.48	11.32	10.90	11.14	11.02	44.86	52.11	48.49	55.15	54.56	54.85
M7	±3.69	±4.02		±1.48	±1.69		±0.50	±0.49		±0.30	±0.63		±1.90	±2.11		±1.74	±1.84	
	160.70	140.17	150.44	127.56	128.01	127.79	12.19	12.09	12.14	9.84	9.67	9.75	48.43	51.03	49.73	51.26	50.53	50.89
MM106	±4.87	±1.47		±1.67	±1.59		±0.19	±0.45		±0.19	±0.21		±1.37	±3.03		±1.35	±1.59	
	107.45	125.63	116.54	146.10	133.75	139.92	9.51	9.17	9.34	9.29	9.40	9.35	44.72	46.56	45.64	49.17	48.53	48.85
M26	±1.91	±2.91		±1.89	±3.56		±0.21	±0.13		±0.24	±0.26		±1.56	±1.90		±2.79	±1.42	
	194.63	178.23	186.43	174.37	166.31	170.34	9.01	9.19	9.10	9.17	9.32	9.25	41.71	39.99	40.85	45.70	44.68	45.19
M9	±2.87	±1.76		±2.09	±2.06		±0.13	±0.15		±0.30	±0.21		±0.77	±0.71		±1.31	±2.24	
Mean	139.18	132.62		134.40	132.01		11.18	11.02		10.33	10.46		46.54	49.66		52.56	53.11	
	Apple rootstocks																	
Rootstock	Total phenolic contents (mg/g DW)						Total free amino acid contents (mg/g)						Peroxidase enzyme activity (EU)					
	Year 2017	Year 2018		Pooled			Year 2017	Year 2018		Pooled			Year 2017	Year 2018		Pooled		
MM111	86.85±1.12	84.30±0.54		85.57			10.16±0.27	10.28±0.58		10.22			54.55±0.74	61.04±1.40		57.80		
M7	100.06±1.22	98.69±1.04		99.37			9.42±0.44	9.60±0.43		9.51			44.30±1.84	48.65±1.04		46.48		
MM106	84.75±1.34	85.26±0.77		85.01			8.99±0.29	8.73±0.13		8.86			47.29±0.67	50.33±1.60		48.81		
M26	94.23±1.08	97.60±1.86		95.91			8.92±0.39	8.81±0.44		8.87			48.58±1.31	48.22±1.25		48.40		
M9	89.74±1.71	92.15±1.27		90.94			8.32±0.44	8.50±0.60		8.41			43.49±1.60	43.81±1.06		43.65		
Mean	91.12	91.60					9.16	9.18					47.64	50.41				

[DW: Dry Weight; EU: Enzyme Units]

99.0 mg/g DW to 186.43 mg/g DW and for Red Fuji varied from 109.59 mg/g DW to 170.34 mg/g DW (Table 3). Both the apple scion varieties grafted on the rootstock M9 recorded maximum amount of total phenolic compounds in comparison when were grafted on the rootstock M7. In contrast to the grafted plants, the results were quite different on the individual rootstocks (Table 3) and it was found that

the rootstock M7 contained the maximum total phenolic content whereas the minimum total phenolic contents were reported in the rootstock MM106 which was found to be closely related to rootstock MM111 and it ranged from 85.01 mg/g DW to 99.37 mg/g DW in rootstocks.

Total free amino acid contents in leaves of both the grafted varieties significantly influenced by the different rootstocks. When Scarlet Gala and Red Fuji were grafted on the MM111 rootstock, contained the maximum amount of the total free amino acid contents i.e, 13.59 and 12.62 mg/g, respectively (Table 3). Whereas, when were grafted on the rootstock M9, contained the minimum amount of the total free amino acid contents i.e., 9.10 and 9.25 mg/g, respectively which was found to be closely related when both the varieties grafted on rootstock M26. Similarly, in case of rootstock (Table 3), the maximum contents of total free amino acid were found in rootstock MM111 (10.22 mg/g) and the minimum contents of total free amino acid in the rootstock M9 (8.41 mg/g) which is also found to be closely related to M26 rootstock.

In the present study, peroxidase enzyme activity significantly affected during grafting. When the Scarlet Gala and Red Fuji were grafted on the MM111 (56.29 EU and 64.39 EU) rootstock (Table 3), they contained the maximum peroxidase enzyme activity whereas, minimum was recorded when grafted on the rootstock M9. While in case of rootstocks (Table 3), MM111 showed the maximum (57.80 EU) peroxidase enzyme activity and rootstock M9 showed the minimum (43.65 EU) peroxidase enzyme activity, respectively.

Molecular characterization

Out of 25 SSR primers, only 16 primers were able to amplify the genomic DNA of stionic combinations of Scarlet Gala, Red Fuji and different rootstocks used in the present study (Table 4, Fig. 1). All the 16 primers were found to be monomorphic in both the varieties. The amplified product was ranged from 50-250 bp for Scarlet Gala and 50-210 bp for Red Fuji grafted on different rootstocks. No polymorphism was obtained with these primers in stionic combinations of both the varieties. Whereas, in case of rootstocks, twelve primers were found to be monomorphic and rest four primers i.e., CH05C02, CH02C02B, CH01H01 and CN918509 were found to be polymorphic, respectively. The CH01H01 primer showed the highest per cent polymorphism i.e. 75%. The CN918509 and CH02C02B showed the 66.70% polymorphism while, CH05C02 showed the 50% polymorphism, respectively. The amplified products were ranged from 50-250 bp. Very low average per cent polymorphism (16.15%) was recorded among the different apple rootstocks used.

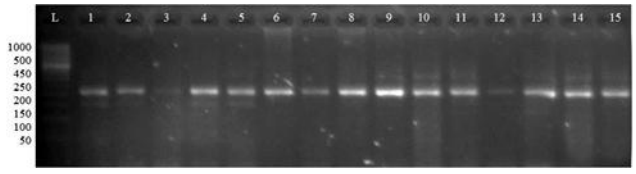

Fig. 1 — Gel showing DNA profiles using SSR marker CN495233: L=50bp ladder; 1-5=Stionic combinations of Scarlet Gala (SG/MM111, SG/MM106, SG/M7, SG/M9, SG/M26); 6-10=Stionic combinations of Red Fuji (RF/MM111, RF/MM106, RF/M7, RF/M9, RF/M26); 11-15=Apple rootstocks (MM111, MM106, M7, M9, M26)

Discussion

Grafting has been used traditionally for vegetative propagation of horticulture and agriculture crops successfully. Despite the wide use of grafting, not much information is available on the graft-induced morphological, biochemical and molecular variations. In the present study, the morphological and biochemical characters were significantly affected due to different rootstocks in both the targeted varieties. The Leaf area, internode length and shoot length of both the varieties significantly affected by the different rootstocks (Table 1). The growth and vigour of the scion could be affected due to the possible reasons: (i) difference in root system activities and the ability of the rootstock's root system to take up minerals and water and their translocation through the rootstock to the scion; (ii) amounts and ratios of the growth promoting or inhibiting endogenous hormones circulating within the tree, particularly between the root system and the aerial tree parts; (iii) movement of assimilates (sugars and amino acids) and mineral elements between the rootstock and scion; and (iv) differences in stem xylem or phloem anatomy and function and the production of inhibitors or the inactivation of promoters within this rootstock, etc. Similarly, in the Red Fuji, the internode length and shoot length showed a high variability when grafted on different rootstocks with a lower value with M9 rootstock as compared to Baleng rootstock, and all others were between these[19].

Rootstocks significantly affected the fruit length, diameter and weight of both the varieties when grafted

Table 4 — Molecular observations on the basis of DNA profiles obtained using SSR markers in different apple stionic combinations and rootstocks

Primer Name	Type	Primer Sequence (5'-3')	Repeat Motifs	LG	Scarlet Gala/Rootstocks							Red Fuji/Rootstocks							Rootstocks						
					Size of amplicons (bp)	No. of bands obtained SG/ MM111	SG/ MM106	SG/ M7	SG/ M9	SG/ M26	Poly-morphism (%)	Size of amplicons (bp)	No. of bands obtained RF/ MM111	RF/ MM106	RF/ M7	RF/ M9	RF/ M26	Poly-morphism (%)	Size of amplicons (bp)	No. of bands obtained MM 111	MM 106	M 7	M 9	M 26	Poly-morphism (%)
CH01B12	F	CGCATGCTGACATGTTGAAT	(AG)20	04	50-150	2	2	2	2	2	0	50-150	2	2	2	2	2	0	50-150	2	2	2	2	2	0
	R	CGGTGAGCCCTCTTATGTGA																							
CH02B03	F	ATAAGGATACAAAAACCCTACACAG	(GA)22	10	50-90	2	2	2	2	2	0	50-150	3	3	3	3	3	0	50-150	2	2	2	2	2	0
	R	GACATGTTTGGTTGAAAACTTG																							
CH05C02	F	TTAAACTGTCACCAAATCCACA	(GA)	ND	180	1	1	1	1	1	0	180	1	1	1	1	1	0	150-180	1	1	2	2	1	50.0
	R	GCGAAGCTTTAGAGAGACATCC																							
CH02C06	F	TGACGAAATCCACTACTAATGCA	(GA)21	02	90	1	1	1	1	1	0	90	1	1	1	1	1	0	90	1	1	1	1	1	0
	R	GATTGCGCGCTTTTTAACAT	(GA)17																						
CH02D12	F	AACCAGATTTGCTTGCCATC	(GA)19	11	100-150	2	2	2	2	2	0	100-150	2	2	2	2	2	0	100-150	2	2	2	2	2	0
	R	GCTGGTGGTAAACGTGGTG																							
CH03D01	F	CGCACCACAAATCCAACTC	GA	02	50-150	3	3	3	3	3	0	50-150	3	3	3	3	3	0	50-150	3	3	3	3	3	0
	R	AGAGTCAGAAGCACAGCCTC																							
CH05D11	F	CACAACCTGATATCCGGGAC	GA	12	250	1	1	1	1	1	0	250	1	1	1	1	1	0	250	1	1	1	1	1	0
	R	GAGAAGGTCGTACATTCCTCAA																							
CH01E01	F	GGTTGGAGGGACCAATCATT	(GT)12A	14	50-120	2	2	2	2	2	0	50-120	2	2	2	2	2	0	50-120	2	2	2	2	2	0
	R	CCCACTCTCTGTGCCAGATC	(AG)32																						
CH01E12	F	AAACTGAAGCCATGAGGGC	(AG)32	ND	200	1	1	1	1	1	0	200	1	1	1	1	1	0	200	1	1	1	1	1	0
	R	TCAATTCACATGAGGCTG																							
CH04E03	F	TTGAAGATGTTTGGCTGTGC	(GA)	05	150	1	1	1	1	1	0	150	1	1	1	1	1	0	150	1	1	1	1	1	0
	R	TGCATGTCTGTCTCCTCCAT																							
CH05E03	F	CGAATATTTTCACTCTGA	AG	02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	R	CAAGTTGTTGTACTGCTC																							
CH01G12	F	CCCACCAATCAAAAATCACC	(AG)45	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	R	TGAAGTATGGTGGTGCGTTC																							
CH03G07	F	AATAAGCATTCAAAGCAATCCG	GA	03	50-150	2	2	2	2	2	0	50-150	2	2	2	2	2	0	50-150	2	2	2	2	2	0
	R	TTTTTCCAAATCGAGTTTCGTT																							
CH04G10	F	CAAAGATGTGGTGTGAAGAGGA	(GA)	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	R	GGAGGCAAAAAGAGTGAACCT																							
CH01H01	F	GAAAGACTTGCAGTGGGAGC	(AG)25.	17	10-100	4	4	4	4	4	0	10-75	3	3	3	3	3	0	10-100	4	3	3	1	4	75.0
	R	GGAGTGGGTTTGAGAAGGTT	5																						
CH01H02	F	AGAGCTTCGAGCTTCGTTTG	(AG)17.	09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	R	ATCTTTTGGTGCTCCCACAC	5 (AG)8																						
CH01H10	F	TGCAAAGATAGGTAGATATATGCCA	(AG)21	08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	R	AGGAGGGATTGTTTGTGCAC																							
CH02C02B	F	TGCATGCATGGAAACGAC	-		50-100	2	2	2	2	2	0	50-100	2	2	2	2	2	0	50-100	2	1	1	3	1	66.7
	R	TGGAAAAAGTCACACTGCTCC																							
CN495233	F	AAGGAGAGAAGAGAGGGAGGA	(TA)9		210	1	1	1	1	1	0	210	1	1	1	1	1	0	210	1	1	1	1	1	0
	R	CATCAAGCGAGGTTCTGACA																							
CN871441	F	AGTCTGGTCAAAACGCAACC	(GA)16		50-190	2	2	2	2	2	0	50-190	2	2	2	2	2	0	50-190	2	2	2	2	2	0
	R	GCTCGGTGCATATAGAAGGC																							
CN908484	F	CAGGCGCCATTTTTAGAGAG	(AG)12		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	R	GGAGTGGCGAATTAGCTGAG																							
CN917587	F	CAAATTCCAAAACTCCCACG	(TCC)6		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	R	GCTTGTAGGACTCGAGGACG																							
CN918509	F	CAACAGTCTCACGCCAAGAA	(CT)10		100-150	2	2	2	2	2	0	140	1	1	1	1	1	0	50-150	2	2	3	2	1	66.7
	R	GGGTGGCGAATCTAAAGACA																							
CO414802	F	AACAGGAGATGGTGGTGGTG	(GGA)7		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	R	TTCGAGATGGGAAATGGAAG																							
CO416273	F	CAAAAATCCAGAATACTCTC	(CT)9		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	R	TCCTCGAGATTTTTCACGCT																							

[SG: Scarlet Gala; RF: Red Fuji; bp: Base Pair; LG: Linkage Group]

on different rootstocks during present investigation (Table 2). This might be due to the root distribution and the timing of phases of active growth that affects water and mineral uptake and hormone synthesis, while differences in root and stem anatomy may affect the translocation efficiency of water, minerals and more complex molecules (carbohydrates and plant hormones) in the xylem and phloem as in case of growth and vigour of scion wood. Further, rootstock can also delay scion vegetative budburst and the onset of shoot extension in the spring, influence the rates of shoot extension throughout the season, affect the timing of seasonal termination of extension shoot growth in the late summer or autumn, influence the branching habit and channeling more of the tree's assimilates and minerals into fruit than into shoot growth. Inconsistent effects of rootstocks on the fruit size across different production area were reported[20]. Similarly, the rootstocks significantly affected mean fruit weight and the highest mean fruit weight was measured when scions were grafted on less vigorous rootstocks such as M7 and MM106 and the lowest on seedling[21]. In contrast, to the present studies, the fruit quality and fruit size of the 'Gala' apple did not influence by the rootstock but it was influenced by the crop load not by the rootstocks[22].

Since in the present study, it was reported that rootstocks did not affect the fruit shape and fruit colour of both the varieties to higher extent (Table 2) but in contrast, the significant effect of the rootstocks on the skin colour of cherry has been reported[23]. Significant difference was observed in the total soluble solids of both the varieties grafted on different rootstocks (Table 2). Similarly, in citrus, scion-rootstock interaction significantly affected the TSS, TA, their ratio and vitamin C levels[24]. Whereas, the effect of seedling, M7, M26 and EMLA has been reported on the soluble solids content and fruit firmness of the cv. Starkspur Supreme Delicious on several sites and concluded that the effects of rootstock on soluble solids content and fruit firmness varied dramatically from site to site[25].

The total phenolic compounds, total free amino acid contents and peroxidase enzyme activity got affected in both the grafted varieties (Table 3). Chemical composition, especially the presence of phenolic compound has been widely investigated in fruits[26], but there is much less information related to content of phenols in the leaves of apple. The presence of phenolic compounds in the leaves of apple may lead to the use of this raw material as a medicine. The total amount of the phenolic compounds in the ethanol extracts of the apple leaves varied from 98.81±1.51 mgGAE/g DW (cv. Aldas) to 163.35±4.36 mgGAE/g DW (cv. Aldas)[27]. Amino acids are building blocks for the synthesis of proteins, enzymes including antioxidant enzymes, provide resistance against stress and regulate the transport of ions and stomata apparatus. The different rootstocks significantly influenced the total free amino acid contents in the grapevines and it was reported to be varied from 945.15 ppm to 1146.15 ppm[28]. Similarly, the rootstock effect was also found significant for free amino acids contents in Chardonnay grapes and it was observed that these contents were found to be lowest when grafted on 140 Ru and 101-14 rootstocks as compared to Schwarzman and K51-40 rootstocks, respectively[29].

Since there is no such literature available that could reveal the reasons behind graft-induced variations in different stionic combinations at DNA level in apple. But there are some reports like in mungbean which reported the significant difference between the scion and that variation was recognized by RAPD analysis[30]. Whereas, the rootstock specific randomly amplified polymorphic DNA markers in the scion, suggesting DNA transfer from the rootstock to the scions were detected[31] but there was no as such DNA transfer reported in the present study. Likewise, the PCR amplification carried out by using ISSR markers reported that the scion fruits had genetic profiling more similar to scion genetic profiling and indicated that minor changes occurred in scion during grafting but not as such report was found using SSR markers[32]. SSR markers have been successfully reported for assessing the genetic diversity and interrelationships in other fruit crops like kinnow[33], mango[34], strawberry[35] and melon[36].

Epigenetic changes like DNA methylation, histone modification, and the influence of small RNA molecules control chromatin pattern, changes in gene expression and cellular function, etc. in plants[37]. There are few reports based on RNA transmission and DNA methylation that may explain the reason behind such graft-induced variations. It has been demonstrated that grafting is getting affected through genetic exchange across grafting junctions between scion and rootstock. Moreover, the evidences behind

substantial changes in DNA methylation in grafted scions might be also due to epigenetic regulations[38]. The significant increase in global DNA methylation in cucumber and melon scions may be due to the epigenetic effect in Cucurbitaceae heterografting[39]. The grafting-induced DNA methylation may contribute to phenotypic variations induced by grafting between tuber mustard and red cabbage[40].

The roles of small RNAs in various plant development processes such as development of leaf, identification of flower organ and flowering transition, etc. have been well demonstrated in Arabidopsis, tomato and rice model plants[41]. The change in small RNA expression induced by grafting which may be an important factor for introducing graft-induced genetic variations in *Brassica juncea* and *Brassica oleracea*[42]. Likewise, the transport of siRNAs from transgenic rootstocks to non-transgenic scion has reported in cherry[43]. More than 3,000 genes transporting mRNAs across the graft junctions have been identified in grapevines[44].

Differential gene expression studies using transcript profiling strategy in the scions of cherry[11], apple[12], sweet persimmon[45] and arabidopsis[46] demonstrated up and down expression levels of genes involved in various cellular development processes which could contribute towards the regulation effect of rootstock in scion growth and development. As graft induced variations holds significant agronomical and horticultural importance towards sustainable crop production, therefore, the selection of potential rootstock genotypes especially in *Malus* species[47] in varying climatic conditions is of great concern and reasons behind such variations along with graft compatibility issues should be addressed on scientific scales significantly.

Conclusion

In the present study, it could be revealed that morphological and biochemical based variations were possibly due to the graft-compatibility, water and nutrients absorption capacity of the rootstock, translocation of water and nutrients through graft-union to the scion and genetic make-up of the type of rootstock used. Though, there was no genetic difference observed among the apple stionic combinations when grafted on different rootstocks using SSR markers. Furthermore, there was no translocation of any genetic loci observed from rootstock to scion wood from the DNA fingerprints derived under each molecular marker analysis. Therefore, from the present findings, it was concluded that rootstock may influence morphological and biochemical parameters of the scion during graft-union but it does not influence genetic constitution of the scion at DNA level. Therefore, keeping in view the above considerations, studies at RNA or protein level are required to unravel the reasons behind these variations.

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Conflict of interest

Authors declare no conflict of interests.

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