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Effect of rootstocks and soil management on growth and physiological parameters in new plantations of apple under replant conditions

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ABSTRACT

Apple orchards planted in late sixties in Himanchal Pradesh and North Western Himalayan region have shown symptoms of declining productivity as these plants have completed their economic life span. Due to limited land and choice of crops for smaller micro climatic niches and incomparable economic equivalence of other fruits with apple, orchardists are compelled to replant at old apple orchard sites. Standardization of suitable agro-techniques to combat replant problem in apple for better field survival rate and productivity is required to sustain apple industry in the state. In the present study, there were 20 treatments comprising of four apple rootstocks i.e. seedling, M793, MM111 and M7 and five different soil management treatments i.e. control, soil fumigation, Plant growth promoting rhizobacteria (PGPR), biocontrol and combined soil fumigation + PGPR + Biocontrol with three replications. The pooled data over the years 2015 and 2016 revealed that M793 rootstock had the maximum growth, vigour, chlorophyll content and photosynthetic efficiency. Among the soil management treatments, the highest growth and vigour parameters, chlorophyll content and photosynthetic efficiency were recorded maximum in combined treatment. The interaction between rootstocks and treatments revealed that combinations of M793 and combined treatment resulted the maximum growth and vigour traits, chlorophyll content, rate of photosynthesis, transpiration rate, stomatal conductance, water use efficiency and minimum stomatal resistance compared to other rootstocks and treatment combinations under replant situations, which can be exploited for the management of replant problem in apple.

Keywords: *Malus* × *domestica*, PGPR, replant problem, rootstocks, *Trichoderma viride.*

INTRODUCTION

Apple (*Malus* × *domestica* Borkh.) a member of family Rosaceae and subfamily Pomoideae, is an important fruit crop of temperate region. In India, apple is mainly grown in North Western Himalayan region which include states of Jammu and Kashmir, Himachal Pradesh, Uttrakhand, North Eastern hilly states and Nilgiri hills, overpaying an area of 2,77,000 ha with annual production of 22,42,000 MT and productivity of 8.0 MT (Anonymous, 1). Himachal Pradesh is known as an "Apple State" of the India because its cultivation has revolutionized the socio-economic condition of farmers and plays a pivotal role in the economy of growers. It is grown over an area of 110, 680 ha with annual production of 4,92,100 MT and productivity of 7.02 MT in the state (Anonymous, 1). Although the area and production under apple cultivation is increasing every year, but the productivity being static and is quite low as compared to other apple growing countries.

Apple plantation of late sixties have shown symptoms of declining productivity owing to various biotic and abiotic factors. With increasing proportion of declining orchards, decreasing land resources due and adverse environmental factors, there has been tremendous pressure on improving production technologies to increase productivity. Due to limited land and choice of crops for diversification in hill states, orchardists prefer to replant old apple orchard sites, with apple which lead to drastic economic loss not only due to uprooting of old trees but also because of poor establishment of new plantations on the same site. Repeated cultivation of the same plant species on the same field is the primary factor leading to replant problems (Singh and Sharma, 13). As a result, a general decline in the growth and productivity of replanted apple orchard is observed. Symptoms include death of fine feeder roots, stunted growth above-ground and below-ground and reduced fruit yield. In most situations, biotic factors have been primarily implicated in apple replant disease, with soil-borne fungi, bacteria, nematodes, actinomycetes and oomycetes variously cited as causal pathogens in site-specific combinations (Mazzola, 9). Replant problem have reportedly been more severe in old tree rows than in the grass lanes of previous orchards. After several years, trees may recover from the initial growth depression and eventually reach the size and annual yields of unaffected trees. Despite this partial recovery, cumulative yields and profitability in ARD-affected orchards usually remain lower than in unaffected orchards (Peterson and Hinman, 11).

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There has been substantial increase in the proportion of declining orchards which need to be changed. Therefore, standardization of rootstocks and suitable soil management treatments to combat replant problem in apple for better survival rate field and productivity under replant conditions was undertaken for sustainability of apple industry in the state.

MATERIALS AND METHODS

The present investigation was carried out on farmer field at an elevation of 2040 m above mean sea level at with location 30°54'N latitude and 77°19'E longitude near village Habban district of Sirmaur (Himanchal Pradesh) on replanted apple orchard site under rainfed conditions during the year 2015 and 2016. The pits were drenched with 10 liters of formaldehyde solution (1:9) after filling the pits. The pits were covered with polythene sheet for three weeks to avoid leakage of formaldehyde fumes. After three weeks the polythene sheet was removed and basin soil was worked in such a way to exclude fumes of formaldehyde from the basins. After two weeks polybag raised clonal rootstocks and seedling were then planted in the pits. Four rootstocks *i.e.* M793, MM111, M7 and seedling and five soil management treatments *viz.*, control, soil fumigation (with formaldehyde), Plant growth promoting rhizobacteria (*Bacillus licheniformis* CK-1), biocontrol (*Trichoderma viride*) and combined (Soil fumigation + PGPR + Biocontrol) were chosen for experiment. Plant Growth Promoting Rhizobacteria [(PGPR) 10^8 CFU/gm minimum of 250 ml] and biocontrol [(*Trichoderma viride*) 10^9 CFU/gm minimum of 10 gm] were applied at the time of planting in pits and then repeated after every three months up to December 2016. Observations regarding growth parameters, *viz*. plant height, stem diameter, number of feathers, leaf area, internodal length, number of nodes, plant spread, plant volume and TCSA were recorded according to standard procedures, chlorophyll content by Hiscox and Israelstam (6) while rate of photosynthesis and transpiration rate with LICOR-6200 portable photosynthesis system during both the years of study. The data on plant growth and physiological traits of apple plants to determine the significance of differences were analyzed by using Randomized Block Design (RBD) with three replication. All data were subjected to two way factorial ANOVA carried out using SPSS computer package (SPSS Inc. USA).

RESULTS AND DISCUSSION

The data on various growth and vigour traits of replanted apple plants presented in Tables 1 to 3 reveal that different rootstocks and treatments exerted significant influence on plant height, stem diameter, number of feathers, leaf area, internodal length, number of nodes, plant spread, plant volume and TCSA compared to control. Among the rootstocks, M.793 rootstock recorded the maximum plant height (170.94 cm), stem diameter (17.88 mm), number of feathers (2.88), leaf area (39.03 cm^2), plant spread (0.830 m^2), plant volume (7.31 m^3) and TCSA (2.56 cm^2) compared to control which was however, the minimum in respect of plant height (150.95 cm), stem diameter (16.33 mm), number of feathers (2.42), leaf area (35.60 cm^2), plant spread (0.751 m^2), plant volume (5.12 m^3) and TCSA (2.16 cm^2) in seedling rootstock. The maximum internodal length (18.35 mm) was recorded in seedling rootstock, whereas the minimum internodal length (16.57 mm) in M.7 rootstock. The highest number of nodes (148.24) was recorded in M.7 rootstock, whereas lowest number of nodes (115.68) in seedling rootstock. Among the treatments, plant height (201.30 cm), stem diameter (19.01 mm), number of feathers (3.57), leaf area (44.69 cm^2), internodal length (21.44 mm), numbers of nodes (142.87), plant spread (1.00 m^2), plant volume (12.03 m^3) and TCSA (2.88 cm^2) were recorded maximum in combined treatment, compared to other treatments which however, recorded minimum plant height (137.11 cm), stem diameter (15.59 mm), number of feathers (1.95), leaf area (32.64 cm^2), internodal length (14.91 mm), plant spread (0.583 m^2), plant volume (2.63 m^3) and TCSA (1.95 cm^2) in control which numbers of nodes (131.26) in soil fumigation. The interaction between rootstock and treatment combinations revealed that M.793 × combined treatment recorded maximum plant height (288.52 cm), stem diameter (19.45 mm), number of feathers (4.06), leaf area (47.12 cm^2), plant spread (1.040 m^2), plant volume (15.18 m^3) and TCSA (3.01 cm^2) compared to other rootstock × treatment combinations. The minimum plant height (130.73 cm), stem diameter (13.18 mm), leaf area (30.88 cm^2), plant spread (0.550 m^2), plant volume (2.31 m^3), TCSA (1.38 cm^2), or number of feathers (1.75) and internodal length (13.11 mm) in M.7 × control while number of nodes (109.54) in seedling × soil fumigation treatment.

The increased plant growth on the rootstocks M793, M111 and M7 in the old declining apple orchard compared to seedling was found to be similar to the findings of Buszard and Jensen (3) which reveal ARD was more severe in soil samples collected from under the canopies than in those taken from the alleyways. The growth of the rootstocks CG30 and CG210 were found similar in both the cases as these two rootstocks had been rated as relatively tolerant to ARD. Seedling rootstocks were found

Table 1. Effect of different rootstocks and soil management treatments on plant height, stem diameter and number of feathers of apple plants at replanted site.

Rootstock / Treatment	Plant height (cm)					Stem diameter (mm)					Number of feathers				
	Seedling	M793	MM111	M7	Mean	Seedling	M793	MM111	M7	Mean	Seedling	M793	MM111	M7	Mean
Control	130.73	143.62	139.72	134.37	137.11	13.18	16.71	16.43	16.06	15.59	2.01	2.01	2.03	1.75	1.95
Soil fumigation	135.87	149.67	147.32	144.17	144.25	14.48	16.87	16.31	16.45	16.03	1.78	2.01	2.38	2.32	2.12
Plant growth promoting rhizobacteria	156.37	168.98	167.77	157.53	162.66	17.53	18.53	17.96	17.86	17.97	2.46	2.79	2.61	2.67	2.63
Biocontrol	149.62	163.92	159.12	153.57	156.55	17.48	17.86	17.70	17.59	17.66	2.64	3.26	2.74	2.65	2.82
Combined	182.17	228.52	206.67	187.87	201.30	18.98	19.45	19.01	18.61	19.01	3.23	4.06	3.58	3.40	3.57
Mean	150.95	170.94	164.12	155.50	160.38	16.33	17.88	17.48	17.31	17.25	2.42	2.83	2.66	2.56	2.62
	CD(0.05)					CD(0.05)					CD(0.05)				
Rootstock (R)	1.01					0.58					0.07				
Treatment (T)	1.12					0.65					0.08				
R×T	2.25					1.31					0.15				

Table 2. Effect of different rootstocks and soil management treatments on leaf area, internodal length and number of nodes of apple plants at replanted site.

Rootstock / Treatment	Leaf area (cm^2)					Internodal length (mm)					Number of nodes				
	Seedling	M793	MM111	M7	Mean	Seedling	M793	MM111	M7	Mean	Seedling	M793	MM111	M7	Mean
Control	30.88	33.71	33.25	32.72	32.64	16.86	16.05	13.61	13.11	14.91	114.88	142.93	144.17	156.47	139.61
Soil fumigation	32.25	33.57	33.40	32.96	33.05	18.33	17.31	15.63	15.61	16.72	109.54	133.54	136.56	145.40	131.26
Plant growth promoting rhizobacteria	36.79	40.64	40.15	37.40	38.74	19.25	18.38	18.72	17.88	18.56	120.17	132.83	142.11	141.95	134.26
Biocontrol	36.07	40.10	38.74	36.04	37.74	18.96	17.95	17.63	16.21	17.69	116.71	137.84	142.49	141.95	134.75
Combined	42.03	47.12	45.51	44.09	44.69	23.16	22.08	20.51	20.01	21.44	117.08	139.42	159.52	155.46	142.87
Mean	35.60	39.03	38.21	36.64	37.37	19.31	18.35	17.22	16.57	17.86	115.68	137.31	144.97	148.24	136.55
	CD(0.05)					CD(0.05)					CD(0.05)				
Rootstock (R)	0.26					0.546					3.348				
Treatment (T)	0.29					0.610					3.744				
R×T	0.58					NS					7.487				

Table 3. Effect of different rootstocks and soil management treatments on plant spread, plant volume and TCSA of apple plants in replanted site.

Treatment \ Rootstock	Plant spread (m^2)					Plant volume (m^3)					TCSA (cm^2)				
	Seedling	M793	MM111	M7	Mean	Seedling	M793	MM111	M7	Mean	Seedling	M793	MM111	M7	Mean
Control	0.550	0.615	0.590	0.575	0.583	2.31	3.01	2.70	2.52	2.63	1.38	2.22	2.15	2.07	1.95
Soil fumigation	0.595	0.690	0.650	0.620	0.639	2.64	3.74	3.31	2.98	3.17	1.68	2.27	2.14	2.16	2.06
Plant growth promoting rhizobacteria	0.843	0.917	0.882	0.858	0.875	5.93	7.66	7.13	6.27	6.75	2.44	2.78	2.57	2.54	2.58
Biocontrol	0.800	0.890	0.845	0.815	0.838	5.03	6.98	6.17	5.57	5.94	2.43	2.54	2.49	2.46	2.48
Combined	0.965	1.040	1.000	0.995	1.000	9.68	15.18	12.58	10.68	12.03	2.89	3.01	2.88	2.75	2.88
Mean	0.751	0.830	0.793	0.773	0.787	5.12	7.31	6.38	5.60	6.10	2.16	2.56	2.45	2.39	2.39
			CD(0.05)					CD(0.05)					CD(0.05)		
Rootstock (R)			0.016					0.22					0.17		
Treatment (T)			0.018					0.25					0.19		
R×T			NS					0.49					NS		

to be more sensitive to replant problem because of their susceptibility to soil borne diseases (Singh *et al.*, 15). Comparatively, the clonal rootstocks (M793, MM111 and M7) were reported to be more tolerant to soil borne diseases Kviklys *et al.* (8) and observed to have more biomass of adventitious roots. Wang *et al.* (17) revealed that *M. hupehensis* showed the maximum tolerance to apple replant disease among all the 5 rootstocks tested. The results of a study on the rootstock performance by investigating root-zone soil microbial consortia and the relative severity of ARD on four rootstock genotypes, showed that the rootstocks M793 and MM111 were relatively tolerant to the ARD compared to all other rootstocks (Singh *et al.* 16). Bhatia and Kumar (2) also reported that apple plants grown on M793 rootstock attained maximum tree growth and vigour. Some of the workers have reported that dwarfing rootstocks CG.5935 (G.935) and CG.4202 (G.202) showed some tolerance to replant disease (Merwin *et al.*, 10). Various studies have shown improvement in plant growth in response to root inoculation with different microbial inoculants capable of producing plant growth regulators (Zahir *et al.*, 18). The enhanced growth of plants may also be attributed to increased nitrogen fixation, phosphate solubilization and increased better utilization of these nutrients in the presence of these rhizobacteria, along with better development of root system and increased photosynthesis. Furthermore, plant growth promoting rhizobacteria and *Trichoderma viride* may also increase plant growth through improvement of the physical, chemical and biological properties of the soil which provide better environment for nutrient uptake and translocation by the plants and enhance production of plant growth regulators such as auxin, gibberellins and cytokines by the plant growth promoting rhizobacteria has been suggested as possible mechanism of action affecting plant growth. The findings are in line with reports of Singh and Sharma (14) who also recorded increased plant height and spread with the application of plant growth promoting rhizobacteria and *Trichoderma viride*.

It is affirmed from the perusal of data given in Table 4 and 5 that the maximum chlorophyll content (2.88 mg^{-1}/g), rate of photosynthesis (8.89 μ $mol^{-1}/m^2/s^{-1}$), transpiration rate (26.74 m $mol^{-1}/m^2/s^{-1}$), stomatal conductance (0.548 m mol/s), water use efficiency (0.332 μ m/mol^{-1}) and the minimum stomatal resistance (0.980 s cm^{-1}) were recorded in plants grafted onto M.793 rootstocks and minimum chlorophyll content (2.75 mg^{-1}/g), rate of photosynthesis (8.19 μ $mol^{-1}/m^2/s^{-1}$), transpiration rate (25.95 m $mol^{-1}/m^2/s^{-1}$), stomatal conductance (0.495 m mol^{-1}/s), water use efficiency (0.315 μ m^{-1}/mol^{-1}) and maximum stomatal resistance (1.150 s

Table 4. Effect of different rootstocks and soil management treatments on chlorophyll content, rate of photosynthesis and transpiration rate of apple plants in replanted site.

Rootstock / Treatment	Chlorophyll content (mg^{-1}/g)					Rate of photosynthesis (μ $mol^{-1}/m^2/s^{-1}$)					Transpiration rate (m $mol^{-1}/m^2/s^{-1}$)				
	Seedling	M793	MM111	M7	Mean	Seedling	M793	MM111	M7	Mean	Seedling	M793	MM111	M7	Mean
Control	2.66	2.81	2.75	2.70	2.73	7.18	8.22	8.26	7.89	7.89	23.36	25.30	24.90	24.23	24.44
Soil fumigation	2.65	2.82	2.79	2.69	2.74	7.49	8.21	8.01	7.88	7.90	25.01	25.60	25.10	24.43	25.03
Plant growth promoting rhizobacteria	2.78	2.88	2.86	2.81	2.83	8.73	9.38	9.14	9.05	9.07	26.70	27.10	26.95	26.84	26.89
Biocontrol	2.76	2.87	2.87	2.80	2.83	8.31	8.93	8.91	8.50	8.66	26.64	27.04	26.89	26.78	26.83
Combined	2.89	3.02	2.99	2.92	2.95	9.24	9.73	9.50	9.38	9.46	28.03	28.65	28.38	28.14	28.30
Mean	2.75	2.88	2.85	2.78	2.81	8.19	8.89	8.77	8.54	8.60	25.95	26.74	26.44	26.08	26.30
			$CD_{(0.05)}$					$CD_{(0.05)}$					$CD_{(0.05)}$		
Rootstock (R)			0.01					0.02					0.07		
Treatment (T)			0.01					0.03					0.08		
R×T			0.02					0.05					0.15		

Table 5. Effect of different rootstocks and soil management treatments on stomatal conductance, stomatal resistance and water use efficiency of apple plants in replanted site.

Rootstock / Treatment	Stomata conductance (m mol^{-1}/s^{-1})					Stomata resistance (s cm^{-1})					Water use efficiency (μ m^{-1}/mol^{-1})				
	Seedling	M793	MM111	M7	Mean	Seedling	M793	MM111	M7	Mean	Seedling	M793	MM111	M7	Mean
Control	0.432	0.482	0.475	0.443	0.458	1.176	1.126	1.150	1.146	1.150	0.307	0.325	0.332	0.326	0.322
Soil fumigation	0.467	0.502	0.494	0.467	0.483	0.982	0.928	0.964	0.955	0.957	0.299	0.320	0.319	0.323	0.315
Plant growth promoting rhizobacteria	0.485	0.527	0.516	0.493	0.505	1.018	0.978	1.012	0.997	1.001	0.327	0.346	0.339	0.337	0.337
Biocontrol	0.517	0.595	0.550	0.529	0.548	1.006	0.965	1.000	0.985	0.989	0.312	0.330	0.332	0.318	0.323
Combined	0.577	0.632	0.622	0.595	0.606	0.957	0.901	0.940	0.919	0.929	0.330	0.340	0.335	0.333	0.334
Mean	0.495	0.548	0.532	0.506	0.520	1.028	0.980	1.013	1.001	1.005	0.315	0.332	0.331	0.327	0.326
			CD(0.05)					CD(0.05)					CD(0.05)		
Rootstock (R)			0.020					0.001					0.001		
Treatment (T)			0.023					0.002					0.001		
R×T			NS					0.003					0.003		

cm^{-1}) in plants raised on seedling rootstock. Among the treatments, highest chlorophyll content (2.95 mg^{-1}/g), rate of photosynthesis (9.46 μ mol^{-1}/m^2/s^{-1}) and transpiration rate (28.30 m mol^{-1}/m^2/s^{-1}), stomatal conductance (0.606 m mol^{-1}/s), water use efficiency (0.334 μ m^{-1}/mol^{-1}) and minimum stomatal resistance (929 s cm^{-1}) were found in combined treatment. The lowest chlorophyll content (2.73 mg/g), rate of photosynthesis (7.89 μ mol/m^2/s), transpiration rate (24.44 m mol/m$^{2/}$s), stomatal conductance (0.458 m mol/s), water use efficiency (0.322 μ m/mol^{-1}) and maximum stomatal resistance (1.150 s cm^{-1}) were recorded in control. The interaction between rootstocks and treatments unveiled that highest chlorophyll content (3.03 mg/g), rate of photosynthesis (9.73 μ mol^{-1}/m^2/s^{-1}), transpiration rate (28.65 m mol^{-1}/m^2/s^{-1}), stomatal conductance (0.632 m mol^{-1}/s^{-1}), water use efficiency (0.340 μ m/mol^{-1}) and minimum stomatal resistance (901 s cm^{-1}) were recorded in M.793 × combined treatment combination which however, recorded lowest chlorophyll content (2.66 mg/g), rate of photosynthesis (7.18 μ mol^{-1}/m^2/s^{-1}), transpiration rate (23.36 m mol^{-1}/m^2/s^{-1}), stomatal conductance (0.632 m mol^{-1}/s^{-1}), water use efficiency (0.340 μ m^{-1}/mol^{-1}) and maximum stomatal resistance (1.176 s cm^{-1}) in seedling × control combinations.

The increase in leaf chlorophyll might be the result of increased leaf area, and balanced nutritional environment in the soil that keep iron physiologically active for chlorophyll synthesis in certain plants. Plants raised on MM.106 rootstocks also had significantly greater leaf chlorophyll content and rate of photosynthesis than those raised on *M. baccata* (Shillong) and seedling rootstocks (Karlidag *et al*., 7), similarly Chandel and Chauhan (4) also recorded greater chlorophyll content in the leaves of plants raised on M9 and MM111 rootstocks and as compared to seedling rootstocks. The increase in photosynthesis, chlorophyll florescence, stomatal conductance and transpiration rate, and decreased stomatal resistance can be attributed to increased leaf area, chlorophyll content and strong source- sink relationship. The results of the present investigation are in accordance with the findings of Rud *et al.* (12) who reported maximum chlorophyll accumulation in the apple trees grown on M.9 rootstock. Godara (5) also observed increased chlorophyll content in plants inoculated with *Azotobacter*as compared to inoculated peach plants. Higher replant resistance with combined treatment may be due to an increase in the net photosynthesis rate which might have caused the increase of relative chlorophyll content and enhancement of maximal photochemical efficiency.

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