DOI : 10.5958/0974-0112.2021.00056.6

Indian J. Hortic. 78(4), December 2021: 392-397

Influence of soil agro-techniques and rootstock on management of apple replant diseases

D.P. Sharma, Niranjan Singh* and K.K. Thakur

Department of Fruit Science, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan - 173230, Himachal Pradesh, India.

ABSTRACT

The replant problem has shown symptoms of declining productivity and longevity of apple orchards in several apple-growing areas of Himachal Pradesh. Due to limited land and choice of crops for smaller microclimatic niches and incomparable economic equivalence of other fruits with apples, orchardists are compelled to replant old apple orchard sites with apples only. Therefore, standardization of suitable agro-techniques to combat the replant problem in apples for better field survival rate and productivity under replant conditions for the sustainability of the apple industry in the state and another part of the country is necessary. The present investigation was carried out using 20 soil agro-techniques combinations, comprising of four apple rootstocks *viz*., seedling, Merton 793, MM.111, and M.7, and five different soil agro-techniques namely soil fumigation, PGPR, biocontrol, combined (Soil fumigation + PGPR + Biocontrol) and control with three replications. The data revealed that Merton 793 rootstock improved the plant growth and vigour parameters, soil enzymatic activities, and microbial counts. Among the soil agro-techniques, similar results were recorded in the case of combined soil agro-technique. Furthermore, the interaction between rootstocks and soil agro-techniques revealed that combinations of Merton 793 × combined technique excelled with respect for growth and vigour traits, soil enzymatic activities, and bacterial, fungal, and actinomycetes counts over other combinations under replant situation.

Keywords: *Malus* × *domestica* Borkh, biocontrol, PGPR, rootstocks, soil biological activities

INTRODUCTION

The apple (*Malus* × *domestica* Borkh.) is mainly grown in North-Western Himalayan region, which includes states of Jammu and Kashmir, Himachal Pradesh, Uttarakhand, North-Eastern hilly states and south Nilgiri hills in India. Apple orchards planted in the early sixties have shown symptoms of declining productivity as these plants have completed their economic life span (Singh and Sharma, 8). Due to limited land resources and choice of crops for diversification in hill states, orchardists are compelled to replant old apple orchard sites with new apple plantation, which lead to the drastic economic loss not only due to the uprooting of old trees but also because of the poor establishment of new plantations on the same site. As a result, a general decline in the growth and productivity of replanted apple orchards is commonly observed.

Apple replants disease (ARD) is a complex syndrome that occurs in young apple trees in replanted orchard sites. Apple replant problem, though reported in the literature for more than century ago, has yet to have its causes clearly defined. The decline in apple productivity has been attributed to the prevalence of fungi, bacteria, nematodes, toxic agents, insect-pests, nutritional disturbances, enzymatic activities and chemical residues. In general, apple orchards of more than 50 years old have shown less fruit yield. There has been increasing concern about poor growth of apple trees replanted at sites where apple tree grew before. The situation resulting in this poor growth is generally known as the replant problem (Singh *et al.*, 9). After several years, trees may recover from the initial growth of depression. Despite this partial recovery, cumulative yields and profitability in ARD-affected orchards usually remain lower than unaffected orchards (Peterson and Hinman, 5). Therefore, there is a need for standardization of suitable agro-techniques to combat the replant problem in apple for better field survival rate and productivity under replant conditions for sustainability of apple industry.

MATERIALS AND METHODS

The present investigation was carried out at the farmer's field at an elevation of 2040 m above mean sea level at location 30° 54'N latitude and 77° 19'E longitude near village Habban district of Sirmaur (Himachal Pradesh) on replanted apple orchard site under rainfed conditions during for two seasons.

*Corresponding author: niranjansinghfruits@gmail.com

The experimental orchard lies under the temperate, sub-humid mid-hill agro-climatic zone III of Himachal Pradesh where, summer is moderately hot (19 and 28 °C) during May-June, and quite severe winter (-1 and 10 °C) during December-January. The annual rainfall ranges between 110-120 cm, and the major amount of which is received during June to September. One-year-old uniform seedling and clonal rootstocks namely, seedling, Merton 793, MM.111, and M.7 were planted in black polythene bags (18" × 9" size) containing a mixture of soil, FYM, and sand (2:1:1). The optimum level of moisture was maintained in the growing medium of polybags by regular irrigation. The suitable methodology has been used to understand the response of apple seedling and clonal rootstocks to replant soil. One-year old polybag raised four rootstocks i.e. Merton 793, MM.111, M.7 and seedling were planted in declining apple orchard site and the pit filled with soil and FYM (3:1) along with soil ball during the first week of January, using five soil agro-techniques *viz*., control (No soil agro-technique), soil fumigation (with formaldehyde), PGPR (*Bacillus licheniformis* CK-1), biocontrol (*Trichoderma viride*) and combined treatment (Soil fumigation + PGPR + Biocontrol). The study was laid out in Factorial Randomized Block Design with three replications. These rootstocks were grafted with 'Super Chief' in March. Plant Growth Promoting Rhizobacteria [(PGPR) 10^8 CFU/g minimum of 250 ml] and biocontrol [(*Trichoderma viride*) 10^9 CFU/g minimum of 10 g] agents were applied at the time of planting in pits and then repeated after every three months up to December. The urease and phosphatase enzymes estimation was carried out by the method described by Tabatabai and Bremner (11). The dehydrogenase and phytase enzymes estimation was carried out by the method given by Casida *et al.* (2). Microbial counts were performed by standard plate counts technique (Wollum, 15) by employing different media for different groups of microorganisms. Observations on tree growth parameters were recorded according to standard procedures by Westwood (14). The leaf area was measured with the help of portable Laser (CI- 202), CID Bio-Science leaf area meter (cm^2). The data on plant growth and soil biological activities of replanted apple were recorded for consecutive 2 years and pooled data were used to determine the test of significance using Randomized Block Design (RBD)-two-way analysis of variance (ANOVA). SPSS program was used to show the interrelationship between rootstocks in combination with soil agro-techniques and mean values of each studied plant growth and physiological parameters.

RESULTS AND DISCUSSION

The pooled data on various soil enzyme, microbial and growth traits of replanted apple plants (Tables 1–6) revealed that different treatments, alone or in combination, exerted the significant influence on soil enzyme activities, soil microbial counts and plant growth characteristics. Plants grafted onto MM.111 rootstock had significantly highest dehydrogenase activity (9.61 μ TPF $g^{-1}h^{-1}$), however, the activities of urease (9.50 μ mol $L^{-1}g^{-1}h^{-1}$), phosphatase (424.25 μ mol $L^{-1}g^{-1}h^{-1}$) and phytase (5186.33 μ mol $L^{-1}g^{-1}h^{-1}$) were maximum in M.793 rootstock. The lowest dehydrogenase activity (9.06 μ TPF $g^{-1}h^{-1}$) was recorded in M.7 rootstock, however, the corresponding values of urease activity (9.05 μ mol $L^{-1}g^{-1}h^{-1}$), phosphatase activity (401.13 μ mol

Table 1. Effect of different rootstocks and treatments on dehydrogenase and urease activities (μ mol $L^{-1}g^{-1}h^{-1}$) of apple plants in replanted site (pooled means for two seasons).

Treatment \ Rootstock	Dehydrogenase activity (μg TPF $g^{-1}h^{-1}$)					Urease activity (μ mol $L^{-1}g^{-1}h^{-1}$)				
	Seedling	Merton 793	MM111	M.7	Mean	Seedling	Merton 793	MM111	M.7	Mean
Control	7.16	7.26	7.28	7.21	7.23	7.19	7.29	7.25	7.23	7.24
Soil fumigation	7.27	7.47	7.91	7.31	7.49	7.28	7.46	7.37	7.33	7.36
PGPR	9.77	10.11	10.28	9.76	9.98	10.00	10.32	9.97	9.99	10.07
Biocontrol	9.51	9.85	10.02	9.50	9.72	9.53	9.85	9.50	9.52	9.60
Combined	12.03	12.21	12.53	11.53	12.08	11.24	12.60	12.24	11.84	11.98
Mean	9.15	9.38	9.61	9.06	9.30	9.05	9.50	9.27	9.18	9.25
		SE			$CD_{(0.05)}$		SE			$CD_{(0.05)}$
Rootstock		0.06			0.12		0.09			0.18
Treatment		0.07			0.14		0.10			0.20
Rootstock × Treatment		0.14			0.28		0.20			0.40

Table 2. Effect of different rootstocks and treatments on phosphatase and phytase activity of apple plants in replanted site (pooled means for two seasons).

Treatment \ Rootstock	Phosphatase activity (μ mol $L^{-1}g^{-1}h^{-1}$)					Phytase activity (μ mol $L^{-1}g^{-1}h^{-1}$)				
	Seedling	Merton 793	MM.111	M.7	Mean	Seedling	Merton 793	MM.111	M.7	Mean
Control	359.02	386.48	379.65	374.02	374.79	4746.50	4911.00	4865.83	4825.83	4837.29
Soil fumigation	382.52	393.49	388.99	386.10	387.77	4806.83	4929.67	4889.83	4871.00	4874.33
PGPR	434.02	456.23	447.15	441.77	444.79	5138.33	5279.33	5198.83	5135.33	5187.96
Biocontrol	390.52	423.49	400.99	393.10	402.02	4987.33	5230.33	5168.83	5009.83	5099.08
Combined	439.60	461.57	454.40	450.52	451.52	5516.17	5581.33	5560.17	5534.17	5547.96
Mean	401.13	424.25	414.23	409.10	412.18	5039.03	5186.33	5136.70	5075.23	5109.33
		SE			$CD_{(0.05)}$		SE			$CD_{(0.05)}$
Rootstock		0.31			0.62		0.83			1.67
Treatment		0.34			0.69		0.92			1.87
Rootstock × Treatment		0.68			1.38		1.85			3.74

Table 3. Effect of different rootstocks and treatments on bacterial and fungal counts of apple plants in replanted site (pooled means for two seasons).

Treatment \ Rootstock	Bacterial count (105 cfu/g soil)					Fungal count (104 cfu/g soil)				
	Seedling	Merton 793	MM.111	M.7	Mean	Seedling	Merton 793	MM.111	M.7	Mean
Control	97.74	98.74	96.41	98.14	97.76	13.22	13.18	13.20	13.20	13.20
Soil fumigation	95.74	96.74	98.64	97.64	97.19	12.72	13.22	14.70	13.20	13.46
PGPR	111.74	114.74	113.64	113.64	113.44	12.72	13.72	13.70	14.20	13.58
Biocontrol	109.24	104.24	104.64	105.64	105.94	13.72	14.22	15.20	14.20	14.33
Combined	116.74	119.74	116.64	118.44	117.89	14.22	15.22	14.70	16.70	15.21
Mean	106.24	106.84	106.00	106.70	106.44	13.32	13.91	14.30	14.30	13.96
		SE			CD(0.05)		SE			CD(0.05)
Rootstock		0.77			NS		0.30			NS
Treatment		0.86			1.75		0.33			0.67
Rootstock × Treatment		1.72			NS		0.67			NS

Table 4. Effect of different rootstocks and treatments on actinomycetes count of apple plants in replanted site (pooled means for two seasons).

Treatment \ Rootstock	Actinomycetes count (10^2 cfu/g soil)				
	Seedling	Merton 793	MM.111	M.7	Mean
Control	14.71	13.71	13.24	13.21	13.72
Soil fumigation	14.21	13.21	13.24	12.71	13.34
PGPR	13.21	12.71	13.24	13.21	13.09
Biocontrol	12.21	13.21	12.21	13.21	12.71
Combined	13.71	12.21	13.21	13.21	13.08
Mean	13.61	13.01	13.03	13.11	13.19
		SE			$CD_{(0.05)}$
Rootstock		0.23			NS
Treatment		0.26			0.53
Rootstock × Treatment		0.52			NS

Table 5. Effect of different rootstocks and treatments on plant height and leaf area of apple plants in replanted site (pooled means for two seasons).

Treatment \ Rootstock	Plant Height (cm)					Leaf area (cm^2)				
	Seedling	Merton 793	MM.111	M.7	Mean	Seedling	Merton 793	MM.111	M.7	Mean
Control	130.73	143.62	139.72	134.37	137.11	30.88	33.71	33.25	32.72	32.64
Soil fumigation	135.87	149.67	147.32	144.17	144.25	32.25	33.57	33.40	32.96	33.05
PGPR	156.37	168.98	167.77	157.53	162.66	36.79	40.64	40.15	37.40	38.74
Biocontrol	149.62	163.92	159.12	153.57	156.55	36.07	40.10	38.74	36.04	37.74
Combined	182.17	228.52	206.67	187.87	201.30	42.03	47.12	45.51	44.09	44.69
Mean	150.95	170.94	164.12	155.50	160.38	35.60	39.03	38.21	36.64	37.37
		SE			CD		SE			CD
Rootstock		0.50			1.01		0.13			0.26
Treatment		0.56			1.12		0.14			0.29
Rootstock × Treatment		1.11			2.25		0.29			0.58

Table 6. Effect of different rootstocks and treatments on number of feather and plant volume of apple plants in replanted site (pooled means for two seasons).

Treatment \ Rootstock	Number of feathers					Plant volume (m^3)				
	Seedling	Merton 793	MM.111	M.7	Mean	Seedling	Merton 793	MM.111	M.7	Mean
Control	2.01	2.01	2.03	1.75	1.95	2.31	3.01	2.70	2.52	2.63
Soil fumigation	1.78	2.01	2.38	2.32	2.12	2.64	3.74	3.31	2.98	3.17
PGPR	2.46	2.79	2.61	2.67	2.63	5.93	7.66	7.13	6.27	6.75
Biocontrol	2.64	3.26	2.74	2.65	2.82	5.03	6.98	6.17	5.57	5.94
Combined	3.23	4.06	3.58	3.40	3.57	9.68	15.18	12.58	10.68	12.03
Mean	2.42	2.83	2.66	2.56	2.62	5.12	7.31	6.38	5.60	6.10
		SE			CD		SE			CD
Rootstock		0.03			0.07		0.11			0.22
Treatment		0.04			0.08		0.12			0.25
Rootstock × Treatment		0.07			0.15		0.24			0.49

$L^{-1}g^{-1}h^{-1}$) and phytase activity (5039.03 μ mol $L^{-1}g^{-1}h^{-1}$) were found in the rootstock seedlings. Of the various treatments, combined treatment tended to show the highest activity of dehydrogenase (12.08 μ TPF $g^{-1}h^{-1}$), urease (11.98 μ mol $L^{-1}g^{-1}h^{-1}$), phosphatase (451.52 μ mol $L^{-1}g^{-1}h^{-1}$) and phytase (5547.96 μ mol $L^{-1}g^{-1}h^{-1}$) enzymes. However, the lowest activity of dehydrogenase (7.23 μ TPF $g^{-1}h^{-1}$), urease (7.24 μ mol $L^{-1}g^{-1}h^{-1}$), phosphatase (374.79 μ mol $L^{-1}g^{-1}h^{-1}$) and phytase (4837.29 μ mol $L^{-1}g^{-1}h^{-1}$) enzymes was recorded in control. The interaction effect of rootstock and soil agro-technique combinations revealed that Merton 793 rootstock and combined treatment had the highest activity of urease (58.75 μ mol $L^{-1}g^{-1}h^{-10}$), phosphatase (461.57 μ mol $L^{-1}g^{-1}h^{-1}$) and phytase (5581.33 μ mol $L^{-1}g^{-1}h^{-1}$), and dehydrogenase activity (12.53 μ TPF $g^{-1}h^{-1}$) in MM.111× combined treatments. However, the activity of these enzymes was observed in seedling × control combination (Table 1 - 2).

Of the various treatments tested in the present study, the bacterial (117.89 10^5cfu/g soil) and fungal (15.21 10^4cfu/g soil) counts were registered to be the highest in combined treatment. However, the lowest bacterial (97.19 10^5cfu/g soil) and fungal (13.20 10^4cfu/g soil) counts were observed in the treatment of soil fumigation and control, respectively. The highest actinomycetes count (13.72 10^2cfu/g soil) was recorded in control, while it was found to be the lowest in biocontrol treatment. Different rootstocks alone or with soil treatment did not show any significant influence on rhizobacterial, fungal and actinomycetes counts. Numerically, all other rootstocks registered higher rhizobacterial, fungal and actinomycetes counts with combined treatment combinations (Table 3 - 4).

The plant growth was significantly influenced by the rootstocks, soil management treatment and their mutual interactions (Table 5-6). Of the four rootstocks, Merton 793 rootstock was found to have maximum plant height (170.94 cm), number of feathers (2.83), leaf area (39.03 cm^2) and plant volume (7.31 m^3) compared to other rootstocks. However, these were minimum in seedling rootstocks. Among the treatments, plant height (201.30 cm), number of feathers (3.57), leaf area (44.69 cm^2) and plant volume (12.03 m^3) were highest in combined treatment as compared to other treatments. However, the lowest plant height, number of feathers, leaf area and plant volume were observed in control. The interaction between rootstock and treatment combinations revealed that Merton 793 × combined treatment resulted the maximum plant height (228.52 cm), number of feathers (4.06), leaf area (47.12 cm^2) and plant volume (15.18 m^3). Minimum plant growth traits were observed in seedling × control. However, the number of feathers was found to be the lowest (1.75) in M.7 × control. The enhanced activity of soil based enzymes at the apple site was observed with the combined application of soil agro-technique and Merton 793 rootstock. Aslantas *et al.* (1) also reported that selected PGPR are able to promote tree growth and reduce the need of chemical fertilizers for young apple trees. The plant growth promoting effect of bacterial applications appeared to be related to phytohormone production and phosphate solubilization activities of the PGPR strains tested.

The soil enzymes are important components of soil health, and their activities are correlated with the soil fertility and efficiency of nutrition to plants. They are important indices for determining the biological activity and productivity of soil (Tuyler, 13). Generally, Pb^{2+} can directly interact with the active functional sites of the enzymes, and change their spatial conformation. The activities of urease are more sensitive to pollution than that of other soil enzymes. The soil enzymatic activities in the planted group increased significantly than those of the control group. When a heavy metal replaces the active functional sites of an enzyme by combining with their mercapto, amino, or carboxyl, the enzymatic activity inhibition would occur, called enzymatic passivation (Zhou *et al.,* 17). Inoculation with AM fungi enriches soil microbe quantities, equilibrates proportion of various microbes, maintains stabilization of proper proportion of the microbes, enhances soil carbon, nitrogen, and phosphorous cycling power, thus improving the activity of soil enzymes (Zhao *et al.,* 16).

Seedling rootstock is more sensitive to replant problem because of their susceptibility to soil-borne diseases. In general, replant sites have more pathogens, thereby, directly affecting the growth and development of new saplings. Comparatively, the clonal rootstocks (Merton 793, MM.111, and M.7) have been reported to be more tolerant to soil-borne diseases (Singh *et al.*, 10), and have more biomass of adventitious roots. Production of plant growth regulators such as auxin, gibberellins, and cytokines by the plant growth-promoting rhizobacteria have been suggested as the possible mechanism of action affecting plant growth. The findings are in line with the report of Thakur (12) who also recorded increased plant height and spread of peach with the application of plant growth-promoting rhizobacteria and *Trichoderma viride*.

Rumberger *et al.* (6) reported that apple rootstock genotype had a stronger effect on the rhizosphere soil microbial community composition than did the pre-plant soil treatments in soils. The plant species-specific rhizosphere microbial communities have been reported widely (Marschner *et al.,* 4) as have changes in rhizosphere microbial communities due to intra-specific variation. In our experiment, the same scion variety ('Super Chief') was grafted onto four different apple rootstocks. The rhizosphere of Merton 793 had the highest culturable soil bacterial counts compared with the other rootstocks, and this rootstock produced the highest plant growth during the course of present studies.

The rootstocks are strongly influenced the composition of rhizosphere microbial colonies. This suggests that rhizosphere fungal and bacterial colonies may be more influential in the promulgation or suppression of apple replant disease (ARD) than pathogenic microbes at this site. These findings are similar to those of Gu and Mazzola (3), who also implicated the involvement of fungi and pseudomonads in ARD. Rootstocks are not only the main factor contributing to observed changes in microbial composition in the rhizosphere, but also a dominant factor for tree growth and yield. Rootstock genotype selection is thus a promising alternative for managing ARD (Shengrui *et al.*, 7). From the present investigation, it can be concluded that Merton 793 and combined technique (Soil fumigation +PGPR + Biocontrol) proved most efficient to influence of soil biological activities and plant growth traits in the apple at the replanted site for effective management of ARD.

AUTHORS' CONTRIBUTION

D.P. Sharma conceptualization the research, executed by N. Singh, K.K. Thakur extended the help in the laboratory analysis and collection of data.

DECLARATION

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

Authors are highly thankful to the facilities and funds provided by Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan 173 230 (H.P.) India.

REFERENCES

1. Aslantas, R., Cakmakc, R., and Sahin, F. 2007. Effect of plant growth promoting rhizobacteria on young apple tree growth and fruit yield under orchard conditions. *Scie. Horti.* **111:** 371-377.
2. Casida, L., Klein, D. and Santoro, T. 1996. Soil Dehydrogenase Activity. *Soil Sci*. **98**:371-6.
3. Gu, Y.H. and Mazzola, M. 2003. Modification of fluorescent pseudomonad community and control of apple replant disease induced in a wheat cultivar-specific manner. *App. Soil Eco.* **24**:57-72.
4. Marschner, P., Yang, C.H., Lieberei, R. and Crowley, D.E. 2001. Soil and plant specific effects on bacterial community composition in the rhizosphere. *Soil Bio. Bio.* **33**:1437-45.
5. Peterson, A.B. and Hinman, R.H. 1994. The economics of replanting apple orchards in Washington state. *Acta Hortic.* **363**:19-23.
6. Rumberger, A., Yao, S., Merwin, I.A., Nelson, E.B. and Thies, J.E. 2004. Rootstock genotype and orchard replant position rather than soil fumigation or compost amendment determine tree growth and rhizosphere bacterial community composition in an apple replant soil. *Pla. Soi.* **264**:246–60.
7. Shengrui, Y., Ian, A.M., George, S., Abawib and Janice, E.T. 2006. Soil fumigation and compost amendment alter soil microbial community composition but do not improve tree growth or yield in an apple replant site. *Soil Biol. Biochem.* **38**:587–99.
8. Singh, N. and Sharma, D.P. 2017. Soil treatments and rootstocks against apple replant problem. *Int. J. Chem. Stud.* **5**:2164-70.
9. Singh, N., Sharma, D.P. and Thakur, K.K. 2018. Effect of rootstocks and soil management on growth and physiological parameters in new plantations of apple under replant conditions. *Indian. J. Hortic.* **75**:392-98.
10. Singh, N., Sharma, D.P., Kaushal, R., Sharma, N., Sharma, I.M. and Sharma S.S. 2020. Isolation and identification of fungi and nematodes in the rhizosphere soil of old declining apple orchards in Himachal Pradesh, India. *Allelo. J.* **50**: 139-52.
11. Tabatabai, M.A. and Bremner, J.M. 1996. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* **1**: 301-17.
12. Thakur, K.K. 2017. Studies on replant problem in peach. PhD thesis, Dr. Y.S Parmar University of Horticulture and Forestry Solan Himachal Pradesh, India.
13. Tuyler, G. 1974. Heavy metal pollution and soil enzymatic activity. *Plant Soil* **41:**303–11.
14. Westwood M.N. 1978. Plant efficiency; growth and yield mesurments. In: Temperate Zone Pomology, W H Freeman and company. *San Fran.* 119-20.
15. Wollum, A.G. 1982. Cultural methods for soil microorganisms. **In***:* Methods of Soil Analysis, Part II, Chemical and Microbiological properties. *Ame. Soc. Agro.* 781-802.
16. Zhao, M., Li, M. and Liu, R.J. 2010. Effects of arbuscular mycorrhizae on microbial population and enzyme activity in replant soil used for watermelon production, International Journal of Engineering. *Sci. Tech.* **2**:17-22.
17. Zhou, L. 1995. Activities of soil enzymes and heavy metal pollution in soil. *J. Env. Sci*. **5**: 176-84.

Received : October, 2019; Revised : August, 2021; Accepted : November, 2021