DOI : https://doi.org/10.58993/ijh/2023.80.2.6

Indian J. Hortic. 80(2), June 2023: 165-170

Scion preconditioning and cytokinin treatment improved graft compatibility in Red Globe grape grafted on Dogridge rootstock

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ABSTRACT

Cultivation of 'Red Globe' grapes are gaining popularity in India's mild tropical climate owing to its bold berries, attractive red colour, and better shelf life, thus fetching a better price in the market. 'Dogridge' is the preferred rootstock in India because of its wide adaptability to different soils and climatic conditions, besides its tolerance to drought and salinity. Though most of the commercial grape varieties are grafted on 'Dogridge' rootstock, the success of grafting 'Red Globe' onto 'Dogridge' is little, or it needs repeated grafting for vineyard establishment. Hence, to improve the grafting success on 'Dogridge' rootstock, we tried different combinations of scion treatments. The scions were treated with cytokinin compounds like kinetin or benzyl amino purine (BAP) or a combination of hormones with scion preconditioning or etiolating and compared with the control. Among these treatments, scions preconditioned on mother vines for five days combined with treating them with BAP @ 100 ppm for 60 seconds before grafting, improved the grafting success to 68%. Biochemical and enzyme studies also indicated higher activity of antioxidant enzymes like peroxidase and polyphenol oxidase in the same treatment. Scion preconditioning and BAP treatment helped better callus production and vascular connectivity between stock and scion, contributing to better graft success. The role of biochemical parameters and antioxidant enzymes in improving graft success has been discussed.

Keywords: *Vitis vinifera*, *Vitis champinii,* benzyl amino purine, *in-situ* grafting.

INTRODUCTION

'Thompson Seedless' and its clonal selections are the major grape varieties commercially grown in India. However, due to their demand in the international market and better price realization, the cultivation of coloured varieties of grapes is gaining importance. The coloured grape varieties, *viz*., 'Flame Seedless', 'Sharad Seedless', 'Red Globe', 'Crimson Seedless' and 'Fantasy Seedless' perform better in the mild tropical climate of southern India than in the major grape growing regions of India, *viz*., Maharashtra and northern Karnataka. In India, the only rootstock which occupies more than 90% of the area under grape cultivation is 'Dogridge' (*Vitis champinii* L.) because of its drought and salinity tolerance (Satisha *et al.,*14). Though it is the most compatible rootstock with many varieties grown in India, it was observed that grafting 'Red Globe' on 'Dogridge' rootstock results in delayed graft compatibility. These necessities repeated grafting to achieve a uniform plant population in the vineyard. Though 'Dogridge' has delayed compatibility with 'Red Globe', it is still the most preferred rootstock due to its commercial advantages in drought and salinity affected areas. Several treatments have been found to improve the grafting success in fruit crops to overcome graft compatibility. However, problems associated with graft union formation can be improved by different treatments. However, with some graft combinations, the graft union appears to be normal, but it is still weak. Further, it is also unclear if the symptoms are associated with delayed incompatibility (Siamak *et al.,*16). Plant growth regulators help improve grafting success by regulating cell division, cell differentiation, and organogenesis. Cytokinins like benzyl amino purine (BAP) and kinetin stimulate rapid proliferation between stock and scion (Kose and Guleryiiz, 9). Preconditioning of scion also improved grafting success in mango, guava, and jackfruit (Akter *et al.,*1). Hence, the study assessed the effect of different scion treatments on grafting success and delayed compatibility in 'Red Globe' grafted on 'Dogridge' rootstock.

MATERIALS AND METHODS

This experiment was conducted at an experimental vineyard of ICAR-IIHR, Bengaluru, Karnataka, during 2018-19. Rooted cuttings of 'Dogridge' rootstocks were planted directly in the field at a distance of 3.3 × 2 m. The plants were allowed to grow for two months with regular cultural operations and later retained 2 to 3 healthy shoots and trained on bamboo poles

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for straight growth. When the shoots attained pencil thickness size (8 mm) after six months of planting, they were *in situ* grafted with 'Red Globe' scions subjected to different treatments.

Scion preconditioning was done by removing the leaf lamina leaving petioles on the shoots five days before grafting on mother vines. On the grafting day, the shoots were detached from mother vines, and used to prepare scions with 3-4 nodes. Etiolation was done by covering the shoots on mother vines with black polythene sheets for five days before grafting, and on the day of grafting, shoots were detached from mother vines and used for preparing scions. As per the treatment, the scions were treated either with BAP or Kinetin alone; preconditioned shoots; etiolated shoots; preconditioned scions treated with BAP@100ppm; preconditioned scions treated with kinetin@100ppm; scions subjected to preconditioning and etiolation together and control.

In situ grafting was performed directly on six month old rootstocks using prepared scions by wedge grafting. Both cut ends of rootstock and scion were first dipped into BAP or Kinetin solution for 60 seconds as per treatment. After dipping, the cuttings were air-dried for 10 minutes and used for grafting. Finally, the stock and scion were firmly secured with 50 µm polyethylene strips. In control, the grafting was done with scions without any treatments. Biochemical constituents in scions and rootstocks were analyzed before grafting using standard procedures for total proteins (Bradford, 4) total phenols (Singleton *et al.,* 17); carbohydrates (Yemm and Willis, 20), nitrogen, CN ratio (using carbohydrate-nitrogen ratio analyzer, Model: VarioMicroCube, Elementar, Germany) and dry matter content. The grafting success, total shoot length, internodal length, and stock-scion ratio were measured 60 and 120 days after grafting (DAG). The whole leaf area per vine was measured on 60th and 120th DAG using a leaf area meter (L 2000, Biovis, Expert Vision Labs, Mumbai) with destructive method. The chlorophyll content (DMSO method), the activity of enzymes peroxidase (POD) (Subhas Chander, 18), and polyphenol oxidases (PPO) (Selvaraj *et al.,* 15) were estimated on 60th and 120th DAG. Histological observations were taken on 120th DAG using scanning electron microscopy (Ensikat *et al.,* 7).

RESULTS AND DISCUSSION

A significant variation in grafting success was recorded following the different treatments (Table 1). The preconditioned scions with BAP@100 ppm gave the highest grafting success of 75.86% and 68.00% at 60 and 120 DAG, respectively. The minimum graft success of 24.14% and 21.43% at 60 and 120 DAG was recorded with preconditioning + kinetin @100 ppm treatments. In control, grafting success of 40.91 and 52.38% were recorded at 60 and 120 DAG, respectively. Similarly, a scion's highest shoot length (153 cm) was recorded with preconditioning + 100ppm BAP on 120th DAG. At the same time, the lowest shoot length (97.00 cm) was recorded in untreated scions. The internodal length between 4th and 5th node was higher in preconditioned scions, treated with 100ppm BAP. A stock-scion ratio of 1.00 was recorded in scions, which received either BAP @ 100ppm alone or in combination with scion preconditioning. The stock-scion ratio (0.92 to 1.06)

Table 1. Effect of various scion treatments on graft success and other growth parameters in Red Globe grapes grafted on Dogridge rootstock.

Scion treatments	Graft success (%)		Total shoot length (cm)		Leaf area per vine (cm^2)		St/scion ratio	
	60DAG	120DAG	60DAG	120DAG	60DAG	120DAG	60DAG	120DAG
BAP	54.55	57.89	69.33	141.57	1971.76	3507.57	0.84	1.00
Kinetin	36.81	36.84	50.00	110.03	1451.62	3411.37	0.90	0.95
Preconditioning	60.00	66.67	49.67	97.00	1855.72	2829.11	0.90	1.06
Etiolation	36.00	31.82	52.00	112.17	1949.76	3069.73	0.94	0.96
Preconditioning + BAP	68.97	68.00	75.67	153.00	2240.62	4937.89	0.95	1.00
Preconditioning + Kintein	20.69	21.43	51.17	121.00	1063.89	2103.58	0.89	0.98
Preconditioning + Etiolation	67.71	63.33	68.00	135.33	2170.94	4057.48	0.88	0.96
Control	45.45	52.38	37.50	97.00	1673.58	2828.94	0.78	0.82
SE (m) ±	2.070	2.191	2.932	2.207	106.741	2.453	0.047	0.082
CD (p≤0.05)	6.340	6.709	8.978	6.758	326.901	283.144	NS	NS

DAG; Days after grafting
NS: Non significant

in the other treatments did not differ significantly. A significant difference was recorded between the total leaf area between the treatments, wherein the scions preconditioned + BAP@100ppm resulted in maximum leaf area (4937 cm^2) followed by the leaf area in preconditioned + etiolated scions (4057 cm^2) per plant. While the least leaf area (2828.94 cm^2) was recorded in the control (Table 1). Many researchers have reported that auxins and cytokinins induce callus proliferation and promote vascular connection by cell division and enlargement (Salisbury and Ross, 13). Many have also reported that treating scions before grafting with auxins or PGPR, which produce auxins, will improve graft union formation by cell division and elongation.

Usually, incompatibility of grafting is failure to form a successful graft union between two plants. However, the delayed compatibility is the very late formation of graft union with a lower percentage of graft success, which has been witnessed in 'Red Globe -Dogridge' combination in many of our previous studies and farmer's fields (Unpublished data). Graft union formation depends on several factors namely rootstock-scion combinations, propagation techniques, environmental conditions, pest and disease incidence, physiological activity of rootstock, and plant growth regulators. Kose and Guleryuz (8) attributed the better callus formation and graft union formation to cytokinin treatment at the cut end of scions just before grafting in grapes. The formation of callus at graft union is one of the prime factors determining good compatibility between scion and rootstocks (Celik, 5), favoring good graft success. The maximum success rate in scions preconditioned and treated with BAP just before grafting may be attributed to the early initiation and proliferation of callus in the graft joint. The preconditioning of scions by removing leaf lamina on mother vines might have activated buds quickly, while BAP is known to be involved in cell division and callus formation. Akter *et al.* (1) reported better grafting success percentage, growth, and development of mango plants when scions were defoliated nine days before detachment. Better graft success in the combination of defoliated scion along with BAP treatment might be due to an immediate rise in sucrose content of phloem sap of shoots which might help in the solute movement towards the shoot apex, resulting in higher meristematic activity at bud level (Maiti and Biswas, 10). The defoliated scions might also have high stored food material, which was visible, as better bud swelling, thus favouring rapid callus formation, and allowing translocation of vital compounds between stock and scion, leading to better graft success. The enhanced callus formation might be the combined effect of scion defoliation and BAP treatment. It is well-established that phytohormones regulate complex physiological relationships between scions and rootstocks. Usually, phytohormones are translocated between stock and scions as signal molecules affecting cell growth and tissue proliferation, especially at the graft union interface (Aloni *et al.,* 2). The auxins and cytokinins are usually prime candidates in scion-stock relationships that transmit signals above and below the grafted region (Kondo *et al.,* 8).Thus, hormonal treatments of the graft union associated with rootstock effects in grapevine are pivotal for the success of the grafting process.

The maximum POD activity at 60 DAG (0.0146 U mg $protein^{-1}$) and 120 DAG (0.0117 U mg $protein^{-1}$) was recorded on vines grafted with preconditioned scions treated with BAP@100 ppm, which was significantly superior to control and was followed by the combination of scion preconditioning and Kinetin @100ppm. Similarly, the maximum PPO activity (0.097 and 0.063 U mg $protein^{-1}$) was recorded in vines grafted with scions preconditioned and treated with BAP@100 ppm, followed by scions treated with BAP @100 ppm alone (0.084 and 0.053 U mg $protein^{-1}$) at the repective days. The least PPO activity was recorded in scions with control (0.033 and 0.013 U mg protein-1 on 60 and 120 days, respectively) (Table 2). Some studies have reported that activation of some of the antioxidant enzymes, *viz*., catalase, peroxidase, and ascorbate peroxidase, in response to the hormonal application, plays a role in graft union formation (Miao, 11). In the present study, we could also observed the higher activity of POD and PPO in scions preconditioned and treated with BAP@100 ppm, with maximum graft success. The treatment of scions with BAP might have caused an increase in peroxidase activity, reported to be located mainly in graft union, was responsible for lignification and resulting in enhanced graft compatibility (Whetten *et al.,* 19). The effect of exogenous cytokinin application on protection by antioxidants has been reported by several workers (Ozden and Karasalan, 12). Among various combinations of scion treatments, preconditioning of scions along with cytokinin resulted in better success with graft healing and proper callusing of tissues either alone or in combination at 120^{th} DAG (Fig.1). At this stage, the continuous callus formation and intermingling of stock and scion callus were observed with the gradual disappearance of the necrotic layer between the two graft components. Anatomical images obtained through scanning electron microscope revealed a more significant portion of the necrotic layer at 60 DAG was eliminated,

Table 2. Effect of various scion treatments on chlorophyll and enzyme activity in grape variety Red Globe grafted on Dogridge rootstock.

Scion treatments*	Total chlorophyll (mgg^{-1})		POD activity (Umg protein^{-1})		PPO activity (Umg protein^{-1})		Shoot dry matter (%)	
	60DAG	120DAG	60DAG	120DAG	60DAG	120DAG	60DAG	120DAG
BAP	1.60	1.99	0.0048	0.0042	0.084	0.053	36.84	40.50
Kinetin	1.31	1.82	0.0031	0.0031	0.061	0.032	29.63	33.97
Preconditioning	1.42	1.85	0.0107	0.0083	0.049	0.039	33.64	37.98
Etiolation	1.07	1.89	0.0062	0.0010	0.011	0.020	30.77	35.43
Preconditioning + BAP	1.76	2.10	0.0146	0.0117	0.097	0.063	38.32	42.32
Preconditioning + Kintein	1.51	1.64	0.0119	0.0113	0.073	0.039	32.90	36.57
Preconditioning + Etiolation	1.40	1.80	0.0035	0.0025	0.054	0.024	36.09	39.75
Control	0.92	1.05	0.0026	0.0022	0.033	0.013	29.27	32.94
SE (m) ±	0.084	0.166	0.00	0.00	0.004	0.002	1.231	1.623
CD ($p \leq 0.05$)	0.256	0.508	0.001	0.001	0.011	0.005	3.769	4.972

DAG: Days after grafting

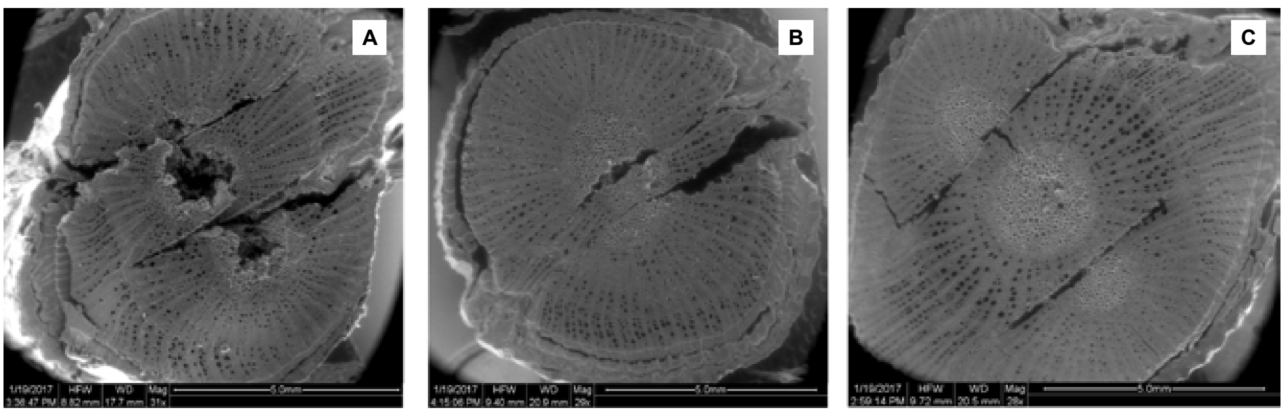

Fig. 1. Anatomy of graft union of Red Globe grafted on Dogridge rootstock with different scion treatments (A). Control, (B). BAP treatment alone and (C). Scion preconditioning + BAP treatmentat 120 days after grafting.

and the grafting union became less distinct by 120 DAG. It is clear from SEM images that the graft union formation has been completed by 120 DAG by continuous ring formation between stock and scion in treatment combination, which received cytokinin in combination with preconditioning or alone as BAP plays an essential role in callus growth by regulating antioxidant enzyme activities and acting as an effective free radical scavenger. The better lignifications and good vascular connection in preconditioned scions treated with BAP might be the reason for better graft success and good scion growth, which might have resulted in increased shoot length, leaf area, and scion girth. The increased total shoot length and leaf area in grafted plants that received combined scion treatment of preconditioning and BAP application (T5) has also been attributed to the good vascular connection between stock and scion portion. Auxins produced at shoot tips affect the production and activity of cytokinins, which are synthesized in roots and translocated to the scion, controlling the primary growth process, such as shoot development and leaf formation (Elfving and Visser, 6). Early formation of graft union in BAP-treated scions might have developed strong communication between auxins produced in shoot tip and cytokinins produced in root tips, which might be necessary for regulating phytohormone balance between root and shoot growth (Bishopp *et al.,* 3).

From the present study, it can be concluded that the problem of delayed compatibility in 'Red Globe' grafted on 'Dogridge' rootstock could be overcome by preconditioning scions for five days before grafting on mother vines, followed by treating the scions with 100 ppm BAP just before grafting. This scion treatment can improve graft success to the extent of 70%, and thus helps to establish a vineyard quickly.

AUTHORS' CONTRIBUTION

Conceptualization of research, supervision of work, manuscript preparation, interpretation of results (JS), treatment imposition, data collection, statistical analysis and collection of review (HP).

DECLARATION

The authors declare that they have no conflict of interest.

ACKNOWLEDGMENT

The authors thank the Director ICAR-Indian Institute of Horticulture for providing field and laboratory facilities for conducting this study. In addition, thanks to the Indian Institute of Science, Bengaluru, for providing facilities to carryout histological studies using scanning electron microscope.

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Received : April, 2023; Revised : June, 2023;
Accepted : June, 2023