Enhancing sapota grafting success: The power of cytokinin application

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Abstract
This experiment aimed to investigate the impact of cytokinin on enhancing the success rate of softwood grafting in the sapota "Cricket Ball" variety conducted at the College of Agriculture, Padannakkad. Various concentrations of cytokinin were applied on different days, with each treatment replicated twice. The experimental factors included cytokinin concentrations (P_1: 100 ppm, P_2 : 150 ppm, P_3 : 200 ppm) and the timing of application: D_1 (on the day of grafting, with cytokinin dipping treatment on the scion), D_2 (5 days prior to grafting, with cytokinin sprayed on the scion), and D_3 (10 days prior to grafting, with cytokinin sprayed on the scion). The results indicated that the treatment of 200 ppm cytokinin applied on the day of grafting (P_3 D_1) was the most effective compared to other treatments.

Key words: Cytokinin, sapota, softwood grafting concentration.

Introduction

The sapota tree, characterized by its evergreen nature and slow growth, produces flowers and fruits at the tips of its branches. Its leaves are elliptical to obovate in shape. The fruits are with thin brown skins, while the seeds are shiny and black. These delectable fruits are packed with essential vitamins such as A, B_1, B_2, B_6 and C as well as minerals like phosphorous, calcium, potassium, iron, sodium and magnesium. The unripe sapota fruit secretes a milky latex known as "gutta percha" or "chuckle," which holds significant market value and can be used as a base material in chewing gum production. Moreover, sapota seeds possess diuretic properties, while the bark exhibits antipyretic qualities.Various processed products, including jam, marmalade, fruit bars, jellies, flakes and dried fruit powders can be derived from sapota fruits. Regarding propagation, although seed propagation is possible, it is time-consuming and results in significant heterogeneity. Hence, vegetative propagation methods such as budding, air layering, side grafting, veneer grafting, approach grafting and softwood grafting are preferred.

Among these, approach grafting has been commonly practiced for many years but is hindered by its costliness, time intensiveness and laboriousness, making it inadequate to meet the substantial demand for planting material. Recently, softwood grafting has gained popularity in the farming and horticulture sectors due to its simplicity, cost-effectiveness and rapid multiplication of plants, boasting a considerable success rate. Studies have shown that the application of cytokinin, a type of hormone, has a positive impact on early graft unions and growth parameters, as evidenced by increased survival rates of grafts and accelerated bud sprouting in grapes under polyhouse conditions (Sunitha et al., 2016). Similarly, the influence of cytokinins on

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grape cutting graft unions has been observed to enhance callus proliferation (Kose and Guleryuz, 2006).

Materials and methods

The aim of the experiment was to assess the impact of cytokinin on the success rate of grafts in softwood grafting of sapota.The present study was performed during the period from November 2020 to June 2021 (off season period) with all required facilities at College of Agriculture, Padannakkad which experiences a humid climate which comes under tropical humid region. It belongs to NARP Northern zone of the state of Kerala and AZ 109th climatic zone of the country. The experiment followed a Completely Randomized Design (CRD) with a factorial approach involving 2 factors. It comprised of 12 treatment combinations, with 15 grafts in each of the 2 replications. Factor 1 focused on the concentration of cytokinin, with four levels: P_1 : 100 ppm, P_2 : 150 ppm, P_3 : 200 ppm and P_4 : Control. Factor 2 involved the timing of application, with three levels: D_1 : on the day of grafting (dipping treatment of cytokinin on scion), D_2 : 5th day prior to grafting (spraying of cytokinin on scion), and D_3 : 10th day prior to grafting (spraying of cytokinin on scion). Scions, selected without any defoliation treatment, were sourced from the mother plant of the Cricket Ball cultivar for softwood grafting. One year old vigorous khirni seedlings of uniform growth were used as rootstock for grafting purpose. Cytokinin treatments at concentrations of 100, 150 and 200 ppm were applied on the 10th, 5th, and 0th day prior to grafting, respectively.

Spraying of cytokinin on the scion was conducted on the 10th and 5th days prior to grafting, while dipping treatment was applied on the 0th day prior to grafting. After grafting, the grafts were placed under a polytunnel (followed by shade net). Observations including the number of leaves per graft, length of scion shoot (cm), leaf area (cm^2), height of graft (cm), girth of stem graft(5 cm graft above graft union) (cm), length and breadth of leaves (cm) and number of successful grafts were recorded at 30, 60, 90, 120, 150 and 180 days after grafting (DAG). Success percentage was noted at 90 DAG, while survival percentage, fresh weight and dry weight were recorded at 180 DAG. Observations were based on the mean values of successful grafts showing uniform growth in each treatment replication.

Results and discussion

Results from experiment are presented and displayed under various headlings.

Number of leaves

There was a notable variance in the number of leaves of grafts concerning the cytokinin concentration and the timing of its application, with significant variations observed between 30 to 180 DAG (Fig.1). The highest number of leaves (22.33) was consistently observed with a cytokinin concentration of 200ppm (P_3) throughout the growth period, except at 60 DAG. Dumanoglu et al. (2014) emphasized the pivotal role of endogenous growth regulators in grafting processes. Cytokinins, classified as N6-substituted aminopurines, instigate cell proliferation in numerous plant cells (Cary et al., 1995). While mature plant cells typically refrain from dividing, this can be induced through the application of plant hormones. The fusion of stock and scion occurs through callus formation on both components. Cytokinins enhance protein levels and stimulate DNA and RNA synthesis in plant tissues, thereby promoting shoot tip growth.

In terms of the individual effect of cytokinin application timing, D_1 - representing treatment on 0thday prior to grafting, indicated highest leaf number from the initial stage to 180 DAG (22.62). Among interaction effects, P_3D_1 - indicating cytokinin at 200ppm + treatment on 0thday prior to grafting - demonstrated the highest leaf count (24.30) compared with all other interaction treatments up to the final growth stage (180 DAG). Conversely, P_4D_3 (18.80), representing the

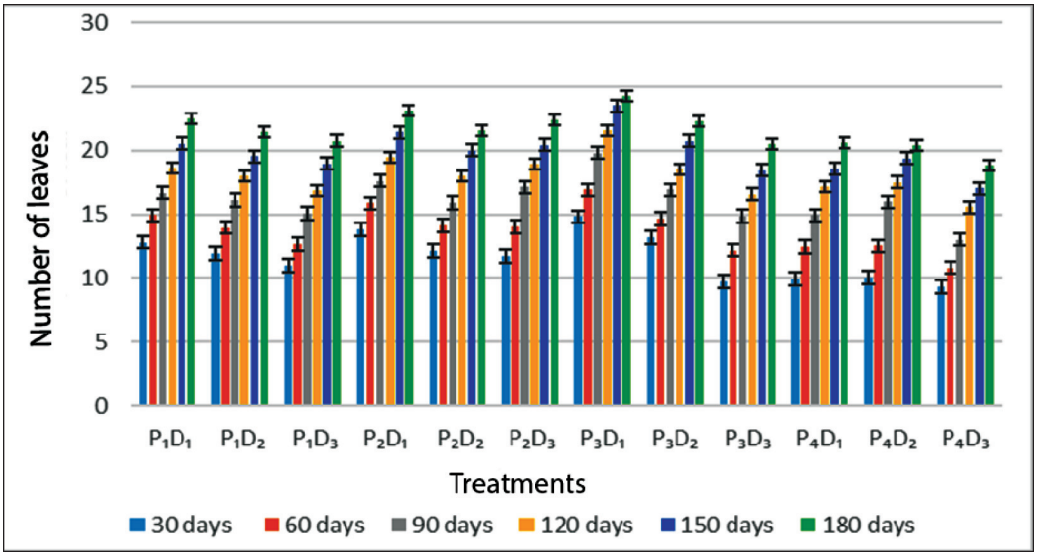

Figure 1. Effect of concentration of cytokinin and days of application on number of leaves

combination of control treatment + treatment 10 days prior to grafting, showed the lowest interaction value among all treatments up to the final growth stage (180 DAG).

Leaf area

The leaf area observation data from 30 to 180 DAG was significantly influenced by varying concentrations of cytokinin and the timings of cytokinin application, both as individual treatments and in interaction treatments (Fig. 2). Analysis of the individual impact of cytokinin concentration on leaf area revealed that the highest value (35.41) was recorded when a concentration of 200ppm (P_3) was applied from the early to the final stages of graft growth. Similarly, among the various timings of cytokinin application, the highest value (33.30) was observed on the 0^{th} day before grafting (D_1), demonstrating its impact on on leaf area throughout the graft growth stages.

Furthermore, considering the interaction treatments,

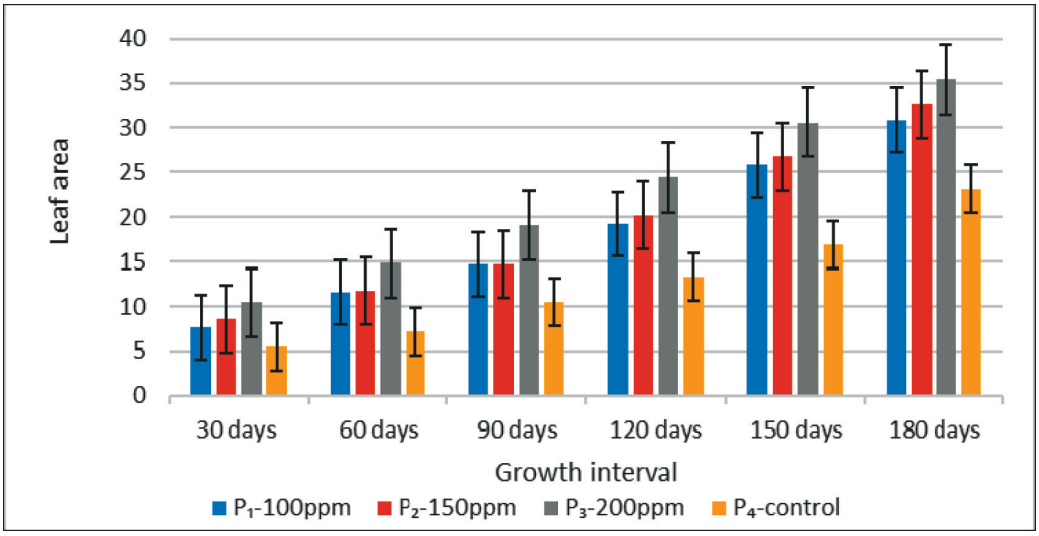

Figure 2. Effect of concentration of cytokinin on leaf area

the combination of cytokinin at 200ppm with application on the 0th day before grafting (P_3D_1) emerged as the most effective treatment, showing a superior value (41.27) compared with other interaction treatments up to 180 DAG. This superior performance may be attributed to the promotion of rapid cell division and elongation facilitated by cytokinin application. These findings align with previous studies by Murashige and Skoog (1962) and Salisbury and Ross (1992).

Length of leaves

The data concerning leaf length indicated variation due to the individual effects of cytokinin concentration and duration of cytokinin treatments from 30 to 180 DAG. The interaction between these factors influenced leaf length from 30 to 150 DAG. The maximum leaf length (12.06) was observed when cytokinin was applied at a concentration of 200ppm (P_3) until 180 DAG. Regarding the individual effect of the duration of cytokinin application, the length of leaves was highest (11.80) on the 0th day prior to grafting treatment (D_1). This could be attributed to the facilitation of water movement by callus formation until vascular connections between the scion and rootstock were established. The application of cytokinins (Kinetin and BAP) promoted callus formation, indicating successful grafting as reported by Hartmann et al. (1997). Among the interaction treatments, cytokinin at 200ppm+0th day prior to grafting (P_3D_1) was identified as the superior treatment (11.82) compared to others up to 150 DAG. However, there were no significant variations among interaction effects at 180 DAG.

Breadth of leaves

Breadth of leaves varied according to the concentration of cytokinin and the timing of cytokinin application. Moreover, the combined influence of both factors affected leaf breadth. The maximum breadth of leaves (4.00) was observed when cytokinin was applied at a concentration of 200 ppm (P_3) from 30 to 180 DAG. The application of cytokinin (kinetin -250 ppm and BAP -250 ppm) significantly enhanced graft take in grapes, as reported by Kose and Muharrem (2006); Sunitha et al. (2016). When considering the individual effects of the timing of cytokinin application throughout the growth intervals, treatment D_1 - 0th day prior to grafting had superior results (3.83). Analysis of interaction treatments on leaf breadth indicated that

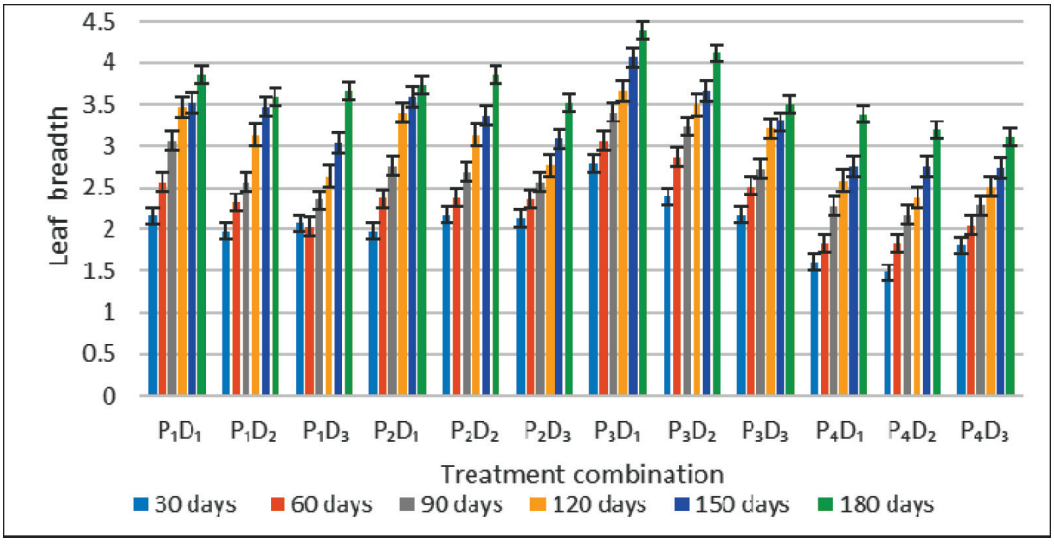

Figure 3. Effect of concentration of cytokinin and days of application on breadth of leaves

the combined treatment of cytokinin at 200ppm + 0th day prior to grafting (P_3D_1) was the most effective (4.39) among all other interaction treatments up to 180 DAG.

Girth of stem

Significant variations were observed in the girth concerning the individual effects of cytokinin concentrations and the duration of cytokinin application, spanning from 30 to 180 DAG. Additionally, an interaction effect was noticed, particularly influencing stem girth from 60 DAG to 180 DAG. Notably, applying cytokinin at a concentration of 200ppm(P_3) to the scion demonstrated superiority at 30, 90 and 120 DAG, while the application of cytokinin at 100ppm (P_1) was found to be more effective at 60, 150 and 180 DAG.

Regarding the individual effects of cytokinin application days, treatment D_1 - applied on the 0th day before grafting demonstrated superior results (3.06) in stem girth throughout the graft growth stages. Analysis of interaction treatments on stem girth suggested that the combination of cytokinin at 200ppm + 0th day prior to grafting (P_3D_1) yielded the most superior results (3.38) up to 180 DAG, except at 30 DAG. This influence might be attributed to the crucial role of cytokinin in the growth of vascular cells during both primary and secondary development of vascular bundles. Furthermore, cytokinin was noted to regulate the growth and development of cambium tissue (Nieminen *et al.,* 2008).

Cytokinin at 200 ppm on the day of grafting found more advantageous for enhancing the biometric parameters of grafts at most of the growth stages. This indicates the high demand of cytokinin during the grafting of sapota and the low endogenous levels. Also immediate availability is also crucial for ensuring the grafting success and further growth. Use of cytokinin during grafting enhance biometric parameters thereby improving the salability of grafts and better field survival.

Conclusion

The findings from our study indicate that applying cytokinin at a concentration of 200 ppm on the day of grafting (P_3 D_1) yielded the most favourable results. Specifically, grafts treated with cytokinin at a concentration of 200 ppm (P_3) demonstrated superior outcomes. Moreover, cytokinin at 200 ppm concentration prior to grafting (D_1) was effective in promoting graft growth. These results suggest that cytokinin application is beneficial for enhancing the growth of softwood grafts of sapota.

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