

Effect of BA on Bud Grafting in Grapefruit

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Abstract: Experiments were conducted at Shambat Research Station, Agricultural Research Corporation, Khartoum North, to test the effect of concentrations and application methods of benzyladenine (BA), as a pre-budding treatment, on scion bud-break and shoot elongation of grapefruit (*Citrus paradisi* Macf. cv. "Redblush"). Nine- to twelve- months old sour orange (*Citrus aurantium* L.) seedlings were used as rootstock. Two application methods of BA (foliar and soaking) were evaluated. The results showed that increasing BA concentration increased all parameters measured relative to the controls, irrespective of the application method. The highest percentage of bud-break, the largest number of leaves, the longest scion shoots and the most growth rating values were obtained with soaking in 400 mg BA/litre treatment, compared with the control or the lower or higher BA concentrations tested or plants treated with the foliar application method. No significant differences were obtained among BA concentrations tested or application methods used on scion shoot length. However, soak application method gave high values for all parameters measured compared with the soak application method. Treatment duration had no effect on the efficiency of BA on bud break.

Key words: Grapefruit; dormant bud; foliar spray; cytokinins; bud-take.

INTRODUCTION

Grapefruit (*Citrus paradisi* Macf.) is a woody rutaceous fruit tree, popular throughout the tropics and subtropics. It is a common citrus fruit tree in the Northern, River Nile, Kassala and Kordufan states, and the fruit is consumed locally as fresh fruit and juice with negligible tonnage exported abroad. "Redblush" grapefruit cultivar is the most widely grown in the country because of its high yields and high quality fruits.

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Eye-budding on a suitable rootstock is frequently the only economical asexual method of propagating citrus, a method that is too slow for producing large number of plants in a short time. Poor bud-break, death and abscission of initiated scion shoots and seasonality of propagation are some of the limitations associated with this method (Nauer and Boswell 1981). Additionally, the rootstock seedlings usually reach suitable sizes for grafting relatively slowly, and a sufficiently long growing season is needed after budding for the budded nursery seedling to reach transplantable size. Tissue culture propagation of citrus, though possible (Ali *et al.* 2004), is economically prohibitive for many commercial citrus growers.

Citruses are perennial, evergreen plants that grow in synchronous flushes. The main flush occurs in February through April. In this regard, citrus nursery men claim that successful scion bud-break in citrus can be obtained only during this time than at any other time of the year. In fact, worldwide research reports support this contention (Nauer *et al.* 1979), where fast and greater scion bud-break is obtained in spring than in autumn. Variation in bud-break across seasons appears to be related to dormancy which occurs annually to varying degrees in citrus trees.

Scion bud-break and subsequent scion shoot elongation in citrus nurseries is most commonly promoted at budding time by the physical removal of the terminal portion of the rootstock seedling, just above the inserted scion bud (topping). The application of this bud forcing method in citrus decreases the photosynthetic capacity of the rootstock and, thus, negatively affects the percentage of scion bud-break and subsequent shoot growth and development (Williamson and Maust 1995). Generally, budded citrus nursery trees grow better when the rootstock shoot remains intact during and after budding than when the terminal portion is removed (Rouse 1988).

There is an enormous demand for nursery budded grapefruit transplants for replanting and establishment of large-scale commercial plantations. Cost of transplants is the largest single factor contributing to total production cost. Thus, there is a need to develop a rapid, cost-effective,

repeatable and easy-to-apply budding procedure for the clonal propagation of elite and desired grapefruit cultivars in large numbers and in short time.

Benzyladenine (BA), a synthetic cytokinin, has been found to have a bud forcing effect on a variety of fruit trees (Williams and Stahly 1968; McCarty *et al.* 1971; Nauer *et al.* 1979; Nauer and Boswell 1981; Boswell *et al.* 1981; Young and Werner 1986; Shaheen and Said 1988). BA is thought to antagonize apical dominance resulting in auxiliary bud-break and subsequent shoot elongation (Erez 1987).

Published information on the use of cytokinins for bud-break in citrus is limited (Nauer *et al.* 1979; Nauer and Boswell 1981). Forcing of grapefruit scion bud-break by BA before excision and budding onto the rootstock will, it is hoped, enhance scion bud-take and promote elongation of scion shoots. Therefore, the objective of this study was to test the effects of BA applied as a pre-budding treatment on scion bud-break and subsequent bud-shoot elongation of grapefruit.

MATERIALS AND METHODS

Experiments were conducted using scions buds obtained from an unpruned, 10-years-old and about 10 metres tall "Redblush" grapefruit tree growing in the open fields of the Department of Horticulture, Ministry of Agriculture and Irrigation, at Al-Mogran (Latitude 15° 35' N; Longitude 32° 33' E). The tree was selected on the basis of its vigorous growth habit and uniformity of fruiting. Sour orange (*Citrus aurantium* L.) stock seedlings, 9-month-old and 60 cm in height, germinated in a lath house from open-pollinated sour orange seeds, were used as rootstock. The seedlings were grown in a soil mix of 1:1 sand: clay in 15 cm diameter black plastic bags (one seedling per bag), watered with tap water as required and no fertilizer was applied.

Benzyladenine, (BA), powder was weighed, using a precision balance, in amounts of 100, 200, 400 and 800 mg. Each weighed amount was dissolved separately in 1N HCl and made to 1000 ml volume with distilled de-ionized water. The solutions, including the controls, contained

1 ml Tween-20 (polyoxyethylene sorbitan monolaurate)/litre as a surfactant. The controls also contained an equivalent volume of 1N HCl.

The selected non-flowering and vigorously growing shoots were sprayed intact on the tree, using a handheld compressed air sprayer, and the appropriate test solution was applied until run-off, in the first experiment. Treated shoots were cut after two weeks from treatment, leaves were acropetally removed and bud-sticks, 10-15 cm in length were tied in bundles and wrapped in wetted newspaper with moist saw dust (the bud-sticks of each treatment being tied and wrapped separately) and were taken to the greenhouse for budding. Similarly, untreated non-flowering, intact and vigorously growing shoots of the same size were cut, tied in bundles and wrapped in wetted newspaper with moist saw dust and were taken to the greenhouse for BA treatments. For each treatment, five bud-sticks were soaked for one hour in the appropriate test solution prior to scion bud excision and budding

The second experiment was conducted to determine the influence of duration of BA treatment on bud-take and scion shoot elongation. Bud-wood was obtained from the same "Redblush" grapefruit tree and was soaked in a solution of 400 mg BA/litre for treatment durations of 1-; 2- ; 3- ; 4- ; or 5-h prior to budding. The 400 mg BA/litre concentration was chosen on the basis of the results of the first experiment where it showed superiority, over the other BA concentrations tested, in increasing all parameters measured.

The T-budding technique was used throughout this study. The inserted scion buds were wrapped with clear plastic tape. The budding process and incubation of budded rootstock seedlings were carried out under naturally lit greenhouse conditions. Budded seedlings were watered on an alternate-day-basis, using tap water, and rootstock suckers were removed as soon as they appeared.

Each treatment consisted of 10 uniform plants arranged in a split-plot randomized complete block design with application methods (foliar or dipping) as the main plots and the BA concentrations as the subplots. Two

control treatments were used for each application method; namely, dry and distilled water spraying on control branches two weeks prior to budding or dry and distilled water soaking of cut bud-wood for one hour before budding. As for the second experiment, a completely randomized design with only one factor (duration of soaking) was used.

Data on the percentage of bud-take, mean average scion shoot length, number of leaves and shoot vigour rating were recorded after 4 weeks from budding. Scion-shoot vigour quality was evaluated visually by 5 panellists in terms of vigorous appearance, leaf development, colour and ease of handling on a scale of 1 to 4, with 1 indicating poor growth and 4 indicating most vigorous growth. The mean values were recorded. Only buds that break and formed a branch were considered to have taken. Scion buds that were swollen and remain green without forming a visible branch were not included in the total count. The data were analyzed using the analysis of variance procedure on Excel computer programme and Duncan Multiple Range Test was used to separate treatment means.

RESULTS

All BA concentrations tested promoted bud-break and increased the number of scion buds that took. The effect of BA on bud-break percentage, number of leaves, scion shoot elongation and quality rating was highly significant. The magnitude of the response of scion buds to break and to grow and develop varied with BA concentrations tested and the method of application employed (Table 1). The most effective concentration (highest percentage of bud-break, longest scion shoots, largest leaf number and the highest quality rate values) was 400 mg BA/litre, irrespective of the application method. Soaking of bud-wood, prior to scion bud excision and insertion into the rootstock in an aqueous solution of 400 mg BA/litre, resulted in significant increases in number of scion bud-break, number of leaves and shoot quality ratings than BA at 0, 100, 200, 800 mg/litre or plants treated with foliar BA sprays. Percentage of bud-break, leaf number and shoot quality ratings were highest at 400 mg BA/litre, decreased at 100 and 200 mg BA/litre and were greatly decreased at 800 mg BA/litre.

Table 1. Effect of BA concentrations and application methods on bud-take percentages, shoot length and number of leaves of budded grapefruit scions, three weeks from budding

Application method	BA conc. (mg /l)	No. of leaves	Shoot length (cm)	Scion shoot quality ^z	Average mean bud-take (%) ^y
Soaking	0.00	8.50c	10.12b	1.2c	10c
	100.00	12.00bc	13.25a	1.6c	50b
	200.00	13.30b	15.40a	3.1ab	41b
	400.00	15.00a	16.67a	3.8a	91a
	800.00	13.50b	13.37a	2.7b	41b
Spraying	0.00	10.01bc	10.00b	1.1c	10c
	100.00	11.00bc	13.01a	1.3c	13c
	200.00	11.31b	15.00a	2.6b	15c
	400.00	12.60b	16.00a	2.7b	33bc
	800.00	12.24b	13.00a	2.5b	18c

Means in a column followed by the same letter (s) are not significantly different at $P = 0.05$, according to Duncan Multiple Range Test.

^zScion-shoot growth rating from 1 to 4, with 1 indicating poor growth and 4 indicating vigorous growth

^yPercent data were transformed to the square root of the arcsine of the proportion for analysis.

All observations were based on 12 budded sour orange rootstocks seedlings per treatment.

The percentages refer to the proportion of the scion buds that took.

Bud-break percentage varied greatly among the individual BA concentrations tested within each application method. The percentage of scion buds which broke ranged from 10% to 33% with the foliar application method and 41% to 91% with the soak method. BA at 400

mg/litre, applied by soaking, gave the largest number of leaves (15.0 leaf/scion shoot) and the control gave the least. The beneficial effects of BA were also reflected on quality rating. The highest values of shoot quality were obtained with 400 mg BA/litre with the soak application method. The least values were, however, obtained with untreated scion buds.

Regardless of the application method, all BA treatments increased scion shoot elongation with significant differences from the control. Although this measure appeared to be unaffected by increasing BA concentrations, higher values were obtained when the chemical was applied by the soaking method than when applied as foliar spray, and non-significantly higher values were obtained with the 400 mg BA/litre treatment than with the other BA treatments.

It is to be noted that bud forcing responses were noticeable on budded scion buds treated with BA concentrations within 7 to 15 days after treatment. A week later, new scion shoots became evident and developed normally. In a few cases, scion shoots were accompanied by secondary shoots beginning to emerge from the same scion bud.

Treatment duration

The results of the experiment conducted to evaluate appropriate treatment duration for maximum percentage of bud-break and production of large number of leaves and long shoots are presented in Table 2. There were no significant differences among treatment durations tested on bud break, leaf number or shoot length. The 2-h treatment duration gave non-significantly high values for all parameters measured, compared with other treatment durations tested. The assessment of shoot quality (in terms of growth vigour and leaf colour and size), however, demonstrated that the 2-h treatment duration gave vigorously growing scion shoots with apparently larger and darker green leaves than those of the other treatment duration tested.

Table 2. Effect of treatment duration on percent bud-take, shoot elongation, and number of leaves of grafted grapefruit scion buds; three weeks from budding

Treatment duration (h)	No. of leaves	Shoot length (cm)	Scion shoot quality ^z	Average mean bud-take (%) ^y
1	9.85a	10.88a	3.3a	85a
2	13.50a	12.33a	3.6a	89a
3	10.33a	12.00a	3.3a	83a
4	12.30a	10.00a	3.5a	88a
5	11.75a	13.12a	3.3a	81a

Means in a column followed by the same letter are not significantly different at P= 0.05, according to Duncan Multiple Range Test.

^z Scion-shoot growth rating from 1-4, with 1 indicating poor growth and 4 indicating vigorous growth

^y Percent data were transformed to the square root of the arcsine of the proportion for analysis.

All observations were based on 12 budded sour orange rootstocks seedlings per treatment.

The percentages refer to the proportion of the scion buds that took.

DISCUSSION

Bud-break was the principal morphogenic pattern in this study. BA treatments were applied to shoot cuttings detached from stock plant containing auxiliary buds, in a similar manner to several investigators (Shimomura and Fujihara 1980; Wainwright and Price 1984; Wang 1987; Svenson 1991). All BA treatments promoted the growth and development of treated buds which normally would remain dormant in general agreement with Shimomura and Fujikara (1980) and Svenson (1991) that BA increases bud-break and growth and development of auxiliary dormant buds of cuttings. Contradictory reports to this finding, however, exist. Skene (1980) and Wainwright and Price (1984) found that BA retards bud growth and development, others reported no effect (Wang 1987; Malik and Archbold 1992) or is phytotoxic (Nauer *et al.* 1979).

Such discrepancies could be attributed to differences in application method, BA concentration, application time, genotype and the physiological stage of bud growth and development.

The enhancement of growth of auxiliary buds in plants by BA is thought to be a consequence of loss of apical dominance as a result of changes in the endogenous balance between naturally occurring hormones (Phillips 1975; Erez 1987). Availability of optimum nutrients and growth promoters to the budded scion bud may also account for the apparent induction of bud-break and subsequent growth. This speculation agrees with Shindy and Weaver (1967) contention that application of BA increased the translocation of assimilates to meristematic regions.

BA significantly increased scion shoot elongation over the control with non-significant difference among BA concentrations or application methods. This result, however, disagrees with those reported by others (Young and Werner 1986; Young 1987; Malik and Archbold 1992) who found that BA has little or no effect on branch elongation. Negative effects of BA on lateral shoot growth and development has been reported (Henny 1985; Wang 1987; Svenson 1991).

Irrespective of the application method used, BA enhanced formation of leaves. The number of formed leaves was positively correlated with BA concentration, with an optimum at 400 mg/litre. This result contrasted the finding of Wang (1987) who reported no effect of BA on leaf number. Differences in results were related primarily to differences in plant genotype, application method, treatment duration and concentration of BA used.

The finding that elongation of the newly formed scion shoot was not affected by increased BA concentration confirmed previous results on navel orange (Nauer *et al.* 1979) and indicated that growth and development that follow bud-break may be under the control of factors other than those optimal for bud-break and growth. A possible explanation for this phenomenon could be that these newly formed shoots lacked some substances necessary for elongation. A report by Malik and

Archbold (1992) supports this contention, where application of BA alone was ineffective in increasing branch elongation. Application of BA and gibberellic acid (GA₃) is essential for shoot elongation. It is also possible that the terminal portion of the rootstock augmented natural apical dominance, hence suppressing scion shoot elongation. This might explain the common cultural practice of removal of the terminal portion of the rootstock by citrus nurserymen, immediately after bud break, to facilitate growth and development of the newly formed scion shoot.

The significantly low values of bud-break obtained with BA foliar spray was, however, some what unexpected. Most earlier reports only involved BA application as a foliar spray (Wilson and Nell 1983; Henny 1985). The response of scion buds to foliarly applied BA concentrations was sporadic and inconsistent, and comparatively low values of all parameters measured were obtained. The highest bud-break percentage (33%) was obtained at 400 mg BA/litre, a value that was less than bud-break percentage recorded at the lowest BA concentration tested with the soak application method. The results of application of BA as foliar spray on scion bud-break reported herein corroborated earlier reports by other investigators (Shimomura and Fujihara 1980; Semeniuk and Griesbach 1985) that BA is ineffective as a dormant bud-breaker when applied to plants as foliar spray.

Direct contact of dormant auxiliary buds with the right concentration of BA (Carpenter 1975) and its absorption by treated buds (Williams and Stahly 1968) are essential for bud-break and shoot elongation. These requirements were satisfied in this study by soaking bud wood sticks, containing dormant auxiliary buds, in aqueous solutions of BA. As a result, significantly high values for all parameters measured were recorded. The soak application method made direct contact of the right concentration of BA with all dormant auxiliary buds, present on the soaked bud-wood stick, and ensured a longer period of wetting at the time of treatment possible and caused most of them to start active growth during the next three weeks following the budding process. This method is more or less similar to previous methods employed for direct application of BA to dormant buds for activation of growth. BA is applied

by dipping in an aqueous solution (Little 1985; Young and Werner 1986), suspended in lanolin paste (Semeniuk and Griesbach 1985), in a saturated cotton swab (Nauer *et al.* 1979) or as foam sprays (Carpenter 1975). All these application methods ensured adequate coverage of buds with the right BA dosage rate and the absorption of BA by the treated dormant buds; thus, the desired responses are obtained.

A single soak application of BA promoted the outgrowth of dormant scion buds and enhanced scion shoot elongation in accordance with the results of Little (1985) and Young and Werner (1986) who found that a single dip treatment is effective in promoting bud-break and growth. Contrary to these results (Little 1984; Henny 1986), higher values of response to BA treatment were obtained with multiple applications of BA than with one or two applications.

The lack of a significant increase in scion bud-break and subsequent shoot growth and development with the foliar spray application method of BA was primarily due to failure to obtain adequate coverage of treated buds and absorption of BA at the time of treatment. Foliage interception and the difficulty of controlling spray drift and drying time made direct contact and absorption of the right concentration of BA by the buds a difficult task. Therefore, the possibility exists for variation in efficacies of BA sprays, depending on the perfection of coverage of the target buds.

BA was effective in activating dormant bud outgrowth only when combined with topping (Carpenter 1975) or lopping (Nauer *et al.* 1979). Uniform, vigorous and normally appearing growth and development of forced budded grapefruit scion buds was obtained in this study without topping or lopping. No phytotoxic effects or deformed growth was observed on forced scion bud or scion shoot, and no shoot or leaf abscission occurred even after the termination of the experiment contrary to the findings of Nauer *et al.* (1979). The results of this study, however, were in general agreement with the findings of Rouse (1988) who obtained better budded citrus nursery trees when the rootstock shoots remain intact during and after budding than when they are physically removed.

The vigorous growth of budded scion buds on intact rootstock, obtained in this study, might be related to a synergistic effect between endogenous growth regulators descending from the upper portion of the rootstock or ascending from the roots and the exogenously applied BA to the scion bud. The rapid formation of vascular connection between the rootstock and the scion bud is vital so that the scion buds may be supplied with water and nutrients from the rootstock by the time the scion bud starts to open. This speculation is in general agreement with the findings of others (Young 1987; Williamson and Maust 1995) that showed that nutrients and growth promoters synthesized by the terminal portion of the rootstock seedling and its roots are essential for forcing bud growth and, consequently, the production of fast and strong growing nursery clonal citrus trees.

Treatment durations seemed to have no effect on the efficacy of BA on bud forcing, bud-take and subsequent growth and development of scion shoots. Treatment durations of less than a minute (Nauer *et al.* 1979; Young and Werner 1986; Wang 1987; Young 1987) have been used for bud-break with varying responses. However, the length of duration of treatments merits further study.

In conclusion, these results attest to the potency of BA as a bud forcing agent and an enhancer of scion shoot elongation in citrus, showing high potential for use in the production of budded citrus nursery trees. The procedure described herein for the clonal propagation of grapefruit gave a higher percentage of bud-break and a greater average scion shoot length without topping, lopping or notching. It is simple, reliable, repeatable, fast and easy to practice and apply and may provide a foundation for development of a general clonal propagation procedure for other citrus species and varieties. Alternative application methods, other types of cytokinins and carriers, application to other citrus species and climatic conditions merit further investigation.

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تأثير البنزاييل أدينين (BA) على التطعيم في القريب فروت

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المستخلص : أجريت تجارب بمحطة ابحاث شمبات- هيئة البحوث الزراعية، الخرطوم بحري، لإختبار تأثير تراكيز وطرق إضافة البنزاييل ادينين (BA)، كعامله ماقبل-التطعيم، لحفز نمو براعم الطعم وإستطالة سيقان القريب فروت صنف "رد بلش" (*Citrus paradisi* Macf. cv. "Redblush").
أستخدمت شتلات لارنج (*Citrus aurantium* L.) بعمر 9 إلى 12 شهراً كأصل للتطعيم، وأختبرت طريقتان (الرش الورقي والغمر) لإضافة ال BA. اوضحت النتائج ان زيادة تركيز ال BA زادت من قيم قياسات كل الصفات تحت الدراسه مقارنة بالشواهد بصرف النظر عن طريقة الإضافة. تم الحصول على اعلى نسبه تفتح لبراعم الطعم واكبر عدد للاوراق واطول سيقان طعم وافضلها نمواً بمعاملة الغمر في محلول الإختبار 400 ملجم BA/ لتر مقارنةً بمعاملات الشاهد او تراكيز ال BA الاعلى او الادنى المختبره او براعم الطعم التي تمت معاملتها بطريقة الرش. لم ترصد فروق معنويه بين معاملات ال BA او طريقتي المعامله المختبره على طول سيقان الطعم، بينما اعطت طريقة المعامله بالغمر اعلى القيم لكل القياسات المرصوده مقارنةً بطريقة الرش الورقي، ولم تؤثر فترة المعاملة على فعالية ال BA كعامل محفز لكسر سكون البراعم.

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