Effect of Type of Wrapping Material and Incubation Conditions on Success of Bud Grafting in Grapefruit (*Citrus paradisi* Macf.)

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Abstract: Experiments were conducted in the nurseries of the College of Agricultural Studies, Sudan University of Science and Technology, Shambat, during the last week of July to the end of October 2006. The aim of this work was to evaluate the effect of different wrapping tapes on budding efficiency and subsequent scion growth and development of budded grapefruit. Buds of grapefruit (*Citrus paradisi* Macf.) cultivar "Redblush" were budded on 12-month old sour orange (*Citrus aurantium* L.) seedlings. The buds were wrapped with one of the following wrapping tapes as treatments: rubberized, cellotape, cellophane, black plastic stripes and electrical tapes. The budded seedlings were placed under three different incubation conditions namely: greenhouse, lath house and outdoor under field conditions. A randomized complete block design was used, with five types of tape as treatments and each replicated eight times with 40 grafted plants placed under each of the three incubation conditions. Parameters measured at the end of the experiment included: percent bud-take, shoot length and number of leaves. The results showed that the type of tape significantly affected percent bud-take, shoot length and number of leaves. Rubberized tape gave the highest values for all measured parameters; cellotape came second, while plastic strips gave the lowest values for all measured parameters. On the other hand, greenhouse conditions resulted in the highest values for all parameters measured followed by the lath house, while the outdoor conditions gave the lowest recorded values.
Keywords*:* Vegetative propagation*, Citrus spp.,* Raffia, Budding, Graft union

Introduction:

Grapefruit (*Citrus paradisi* Macf.) is a member of the family Rutaceae grown for its fruit, which contain relatively less sugar and more citric acid, between 1.5 and 3.0% (Samson, 1986). Grapefruits may be eaten fresh as sweet orange and mandarin, or their segments may be used in fruit salads, or canned. Juice may also be extracted and consumed, or it may be concentrated. Grapefruit is the third most important citrus in the world after orange and mandarins (FAO, 2004) occupying a prominent place in the United States citrus industry used mainly as a breakfast fresh fruit or as juice because of its refreshing flavour and bitterness. In Sudan, grapefruit is a major cash crop and a common component of diet rich in vitamins (Sidahmed and Geneif, 1984_a).

It is one of the most important citrus fruits in Sudan and can be successfully grown throughout the country where there are suitable soils and sufficient water to sustain tree growth (Sidahmed and Geneif, 1984_b). Sudanese grapefruit is well known for its large size, excellent quality and good coloration (Khalil, 1985). Several grapefruit cultivars have been introduced into Sudan and were evaluated for their growth, yield, and fruit quality (Dinar and Osman, 1984; Hamid *et al*., 1999; Sidahmed and Geneif, 1984a). The introduced cultivar "Redblush" proved to be high yielder with excellent fruit qualities and vigorous growth habits.

Citrus trees generally are propagated in most citrus growing areas by budding the scion of the desired variety into a chosen seedling rootstock. The budding process and time of budding vary with locality and proved to be tedious, expensive and need high technical know-how. The percent take is variably low, seasonal and variety dependent.

After bud insertion, the bud union is tied up with different tying materials (Meyer, 1988). Successful budding requires that the bud union be held together until the scion and the rootstock unite. Wrapping with a suitable material is essential to promote healing and prevent drying of the bud union. Various

wrapping materials have been successfully used for that purpose (Hartmann *et al*., 1997).

Soft plastic tape has been found most appropriate for kiwifruit grafting or budding (Zenginbal *et al*., 2006) and plastic strips have been used with success to wrap graft or bud union of a variety of tree species (Beineke, 1978; Hartmann *et al*., 1997). Rubber strips and electric tapes have also been used in Sudan with varying degrees of success.

Due to the high demand for budded grapefruit trees; citrus growers tried to produce their own budded grapefruit seedlings in their orchards. They used different kinds of wrapping materials and tying methods including raffia, rubber bands, banana fibers, soft polyethylene strips, paper tape and cotton yarn. There is no specific mention on how these wrapping materials affect budding success. Hence, the present study was under-taken to examine the effect of different wrapping materials under different environmental conditions on success of grafted grapefruit, with the original aim of developing an easy, simple, repeatable; cost-effective and practical technique for the production of large numbers of budded grapefruit seedlings.

Materials and Methods

The experiments of this study were conducted at the nursery facilities of the Department of Horticulture College of Agricultural Studies, Sudan University of Science and Technology, Shambat, Khartoum North, to evaluate the effectiveness of wrapping materials on success of budding under different incubation conditions. The T-budding method was used throughout these experiments and grapefruit (*Citrus paradisi* Macf.) cv. "Redblush" was used as a source of scions.

The mother tree is a 7-year-old grapefruit tree growing in the orchard of the Horticulture Section Administration, which belongs to the Ministry of Agriculture and Forestry, Khartoum. All bud woods were collected from this single tree. The bud woods were 20-30 cm in length. Undesirable or abnormal wood parts were discarded. The leaves were cut-off leaving a stub of the petiole to protect the buds. Bud sticks were placed in plastic bags containing moistened newspapers to maintain turgidity and freshness and to prevent drying of tissues.

The rootstocks used were one-year-old sour orange (*Citrus aurantium* L.) seedlings obtained from the nursery of the Horticulture Section Administration, Khartoum. Selected rootstocks with uniform thickness and age were transferred and maintained in the lath house of the Department of Horticulture, College of Agricultural Studies, Sudan University of Science and Technology, Shambat, until use. The rootstock seedlings were grown in black polyethylene bags with dimensions of 20x25x30 cm perforated at the basal end to drain excess irrigation water.

The rubberized tape was obtained from the Department of Horticulture College of Agricultural studies, Sudan University of Science and Technology, Shambat. Other wrapping materials including cellophane, cellotape, electrical tape and the black plastic strips were purchased from the local market.

All grafting operations were done in the lath house. Using a sharp budding knife, one cm vertical cut through the bark of the rootstock 20-30 cm above the ground was made. At the upper end of the vertical cut, a horizontal cut was made forming a T-shaped cut. With the budding knife, a bud along with a piece of wood and bark from the wood stick was removed. The bud was inserted under the flaps of the "T" shaped cut of the rootstock in a way that it is completely enclosed by the "T" flap. The bud was wrapped with a budding tape in two or three rounds below and two or three rounds above the bud. The eye of the bud was left free. The terminal shoot tip of the rootstock was cut (topping) leaving few leaves above the grafted bud, a practice that is done routinely in Sudan to enhance bud-take. Grafted seedlings were distributed randomly, 40 seedlings in each of the three incubation conditions i.e. greenhouse, lath house and outdoor. The plants were given equal fertilizer doses and irrigation, and pesticide applications.

One month from budding the presence of a green healthy looking bud indicated that bud-take has occurred. A bud that did not take turns black or brown. After two months, another

topping was made, again 3 to 10 cm above the grafted bud. All suckers produced on the rootstock were removed as soon as they appeared.

After three months from the budding process percenttake, length of new shoots (cm) and number of leaves were recorded. A randomized complete block design was used. Each of the five treatments was replicated eight times and each incubation condition consisted of 40 grafted plants.

Data were subjected to analysis of variance procedures of the Statistical Analysis System (SAS Institute, 1990). Means separation was performed using Duncan's Multiple Range Test at 95% confidence level.

Results and Discussion

Bud-take and types of tape:

Table (1) summarizes the influence of wrapping materials on bud-take under different incubation conditions. Rubberized tape proved to be the best in overall performance giving an average of 75% bud-take, cellotape was second with 66.6% and electrical tape was the third best with 62.5% followed by cellophane tape 58.3% bud- take. Black plastic strips gave the least value of percent bud-take (25%). The superiority of rubberized tape may be attributed to its ability to prevent drying of cut surfaces making good contact by hardly pressing the scion to the rootstock and so promote callus formation; conditions that are promote rapid set of cambial connectivity between the bud and the rootstock. This result is in agreement with a report by Zenginbal *et al*. (2006). The data introduced rubberized tape as a suitable wrapping material for grapefruit and other citrus species since no effect of cultivar on budding efficiency using different types of tape exist (Oliveira *et al*., 2004).

On the other hand, soft-wrapping materials, white or transparent cellot-ape and cellophane performed well on grapefruit budding and gave sensibly high bud-take percent. They are easy to wrap and make more effective contact between the scion bud and the rootstock maintaining good conditions for wound healing by conserving humidity around the bud union and preventing drying of bud and hence increasing bud-take in a very similar manner as reported by Skene *et al*. (1983); and Hartmann *et al*. (1997). Takishita *et al*. (2002) found cellophane tape to be the most effective wrapping material for grafts of citrus relatives. White or transparent plastic tapes were the tying materials of choice for wrapping bud union of kiwifruit (Howard, 1977; Howard *et al*., 19974).

The results with electrical tape however, agree with the findings of Singha (1990), where this tape was found to be effective and useful for graft union formation and was thus recommended by him to amateur horticulturist for use as wrapping material. The results obtained with black plastic strips were unexpected since this wrapping material has its reputation as the most exclusively used tape for tying apple grafts (Larson 1976; Singha, 1990) and air layerings of guava (Patel *et al*., 1996) and papaya (Tawfik, 2002). The low percent bud-take reported here may be attributed to the difficulty encountered with black plastic strips usage. The lack of adhesive might have made it hard to use resulting in improper wrapping of the bud union. Their black colour might have contributed to the decreased budtake since it absorbs more heat especially if tempe-rature degrees are relatively high as the case during our experimentation.

These results, however, disagree with reports that types of tape had no effect on percent bud-take (Singha, 1990; Oliveira *et al*., 2004). Likewise, our results are consistent with those of (Zenginbal *et al*., 2006; Howard, 1977; Howard *et al*., 1974) where the type of wrapping material affected success or failure of bud-take and even the colour of the wrapping material has been found to affect the budding process (Singha, 1990; Zenginbal *et al*., 2006) as well as air layers (Patel *et al*., 1996; Tawfik, 2002).

Bud-take and incubation condition:

Budding success was high in grafts, incubated under greenhouse conditions (72.5%) followed by lath house (55%), while outdoor conditions gave the least percent bud-take (45%) (Table

1). This could be attributed to the increased humidity in the greenhouse, which progressively decreases in the lath house and in outdoor conditions. Relatively high atmospheric humidity reduced evapotranspiration from the budded seedling and minimized evaporation from the planting medium thus favouring the active growth of grafts. Similar observations were reported by Kotalawala (1972) on mango and by Sen and Kapedia (1984) on sweet orange. The importance of keeping grafted plants under high humidity for callus formation and subsequent success of grafts has been advocated by Takishita *et al*. (2002). Relatively low humidity impairs bud-take through its effects on water loss from the cut surface and its prohibitive effect on callus formation and wounds healings.

Hartmann *et al*., (1997) recommended keeping grafts under conditions of relatively low temperature for successful high percent bud-take and subsequent better growth and development of grafts. High temperature increases water loss through transpiration and evaporation leading to failure of bud-take (Tanimoto, 1994).

Shoot length and types of tape:

The data of shoot length measurements showed no significant differences among types of tape tested. Despite this, the highest values of mean shoot length were obtained with rubberized tape (5.86 cm), cellotape (4.51 cm), then electrical tape (3.46 cm), while black plastic strips gave the least value (1.16cm) (Table 2). The data agreed with those of Oliveira *et al*., (2004) who reported lower shoot length when plastic transparent tape was used as wrapping material compared to parafilm degradable tape, but is in conflict with Singha (1990) who reported significant differences between shoot length as a result of type of tape where masking tape gave significantly shorter shoot length than plastic strips or duct tape. This disagreement in results might be due to differences in wrapping tape used, genotype, locality and the time of the year when the budding was done. It could also be attributed to the poor adhesive nature of our black plastic strip and its undesirable tendency to unravel especially during the healing of the graft union.

Shoot length and incubation conditions:

The incubation conditions, however, had influenced shoot length. Significant differences ($P \leq 0.01$) were reported for mean length of shoots under different incubation conditions. The highest mean values of shoot length were obtained under greenhouse conditions with significant difference from the outdoor conditions while the lath house was intermediate.

Shoot length is closely associated with the establishment of cambial connectivity between the rootstock seedling and the budded scion (Skene *et al*., 1983). Cambial connectivity is of utmost importance in allowing for the flow of water and mineral nutrients from the rootstock to the scion. Under favorable environmental conditions of relatively low temperatures and high relative humidity callus formation occurs on cut surfaces and enhances cambial connectivity between the rootstock and the scion bud.

There were no significant differences in shoot length among the wrapping materials under different incubation conditions. The highest mean values of shoot length were obtained with cellotape under greenhouse (8.92 cm) followed by electrical tape also under greenhouse (6.91 cm), rubberized tape under outdoor (6.41 cm) and then rubberized tape under lath house (5.89 cm) conditions.

Number of leaves and types of tape:

Table (3) summarizes the results of the effect of wrapping material on number of leaves. Rubberized tape gave the highest value of number of leaves (7.88) followed by cellotape (5.83), electrical tape (5.67), and then cellophane tape (4.21) with no signifi-cant difference between them. Black plastic strips, however, gave signifi-cantly less number of leaves compared to all other wrapping tapes tested (2.13).

These results concur with data observed in this study for percent bud- number of leaves take

and shoot length where black plastic strips gave the least values for percent bud-take and for shoot length. Black plastic strips do not properly hold the bud union together until the scion and the stock unite, nor does it create conditions suitable for wound healing. Instead it enhances drying in the vicinity of the bud

union and delays callus formation and subsequent cambial connectivity between the scion and the rootstock, necessary for leaves growth and development.

Number of leaves and incubation conditions:

Incubation conditions seem to have no effect on number of leaves per plant. The highest number of leaves was obtained under greenhouse conditions (6.80) with lath house being second (4.45) and the lowest value for was obtained under out-door conditions (4.18) with no significant difference between treatments.

This study presented a procedure for the clonal propagation of the grape-fruit cultivar "Redblush" by budding under greenhouse conditions using readily available wrapping tapes. Further refinement of the procedure is necessary to maximize percent-take and consequently transplant production so that a reliable and cost-effective system for commercial application can be developed. The procedure may provide a foundation for development of a general vegetative propagation system for other citrus species and cultivars.

Table 1. Effect of wrapping materials and incubation conditions on percent bud-take of budded grapefruit scions. Data were taken after three months from budding.

Types of tape	Bud-take (%)			
	Incubation condition			
	Greenhouse	Lath house	Outdoor	Average
Rubberized tape	75.0	62.5	87.5	75.0
Cellophane tape	75.0	62.5	37.5	58.3
Cellotape	100.0	62.5	37.5	66.6
Black plastic strips	37.5	25.0	12.5	25.0
Electrical tape	75.0	62.5	50.0	62.5
Average	72.5	55.0	45.0	

Table 2. Effect of wrapping materials and incubation conditions on shoot length of budded grapefruit scions. Data were taken after three months from budding

Types of tape	Incubation conditions			Mean
	Greenhouse	Lath house	Out-door	
Rubberized tape	5.53	5.89	6.14	5.86
	(2.51)[a]	(3.04)[a]	(2.36)[a]	(2.64)[a]
Cellophane tape	4.70	3.54	0.98	3.07
	(2.53)[a]	(2.20)[a]	(1.81)[a]	(2.18)[a]
Cellotape	8.92	2.79	1.81	4.51
	(2.91)[a]	(2.08)[a]	(2.05)[a]	(2.35)[a]
Black plastic strips	1.81	1.61	0.06	1.16
	(2.26)[a]	(1.68)[a]	(1.18)[a]	(1.71)[a]
Electrical tape	6.91	1.34	2.13	3.46
	(3.08)[a]	(1.41)[a]	(2.01)[a]	(2.17)[a]
Mean	5.57	3.03	2.22	
	(2.66)[a]	(2.08)[ab]	(1.88)[b]	

* Data transformation ( )*, **Means followed by the same letter(s) are not significant different at P=0.05, according to Duncan Multiple Range Test.

Table 3. Effect of wrapping materials and incubation conditions on number of leaves of grapefruit scion. Data were taken after three months from budding

Types of tape	Conditions			Mean
	Greenhouse	Lath house	Outdoor	
Rubberized tape	6.88	7.25	9.50	7.88
	(2.91)[a] *	(3.48) [a]	(2.89) [a]	(3.10) [a]
Cellophane	3.70	3.38	2.25	4.21
	(3.08)[a]	(2.18)[a]	(2.58) [a]	(2.61) [a]
Cellotape	9.00	4.63	3.88	5.83
	(2.97)[a]	(2.66) [a]	(2.85) [a]	(2.83) [a]
Black plastic strips	2.63	3.75	0.00	2.13
	(2.61)[a]	(2.16) [a]	(1.01) [a]	(1.93) [b]
Electrical tape	8.50	3.25	5.25	5.67
	(3.39)[a]	(2.03) [a]	(3.03) [a]	(2.82) [a]
Mean	6.80	4.45	4.18	
	(2.99)[a]	(2.50) [a]	(2.47) [a]	

* Data transformation ( )* , **Means followed by the same letter(s) are not significant different at P=0.05, according to Duncan Multiple Range Test.

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تأثير نوع مادة الربط وظروف الحضانة على نجاح التطعيم بالعين في القريب فروت (*Citrus paradisi* Macf.)

فاطمة دياب أحمد صالح و عبد الغفار الحاج سعيد
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المستخلص:

أجريت هذه التجارب بمشاتل كلية الدراسات الزراعية – جامعة السودان للعلوم والتكنولوجيا بشمبات في الفترة من الأسبوع الأخير من يوليو وحتى نهاية أكتوبر2006م بغرض تقييم أثر أنواع أشرطة الربط المختلفة على كفاءة التطعيم ومن ثم نمو وتطور شتلات القريب فروت المطعومة. تم تطعيم براعم قريب فروت (*Citrus paradisi* Macf.) صنف "ردبلش" على أصول لارنجا (*Citrus aurantium* L.) بعمر 12 شهر. ربطت البراعم على الأصل بعد التطعيم بإستخدام نوع واحد من أنواع الأشرطة التالية: المطاطي،" سليتيب"،" سلفان"،أشرطة بلاستيك اسود وشريط الكهرباء. وضعت الشتلات المطعومة بعد ذلك في كل من البيت المحمى، البيت الخشبي و في الخارج تحت ظروف الحقل. أستخدم تصميم القطاعات الكاملة العشوائية بخمسه أنواع أشرطة مختلفة كمعاملات كررت كل معاملة 8 مرات ووضعت 40 شتلة مطعومة في كل واحدة من أنواع الحضانة الثلاثة المختلفة. شملت القياسات التي رصدت في نهاية التجربة نسبة نجاح الطعم، طول الأفرع، وعدد الأوراق. أوضحت النتائج أن نوع شريط الربط له تأثير معنوي على نسبة نجاح الطعم، طول الفرع وعدد الأوراق، حيث أعطى الشريط المطاطي أعلى قيم لكل القياسات المرصودة وجاء "السلتيب" في المرتبة الثانية بينما أعطت أشرطة البلاستيك أدنى قيم لكل القياسات المرصودة. أوضحت النتائج أيضا أن الحضانة في البيت المحمي أعطت أعلى قيم لكل القياسات المرصودة تلاه البيت الخشبي، بينما أعطى وضع الشتلات في الخارج أقل القيم المرصودة.