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Clonal Production of Cotton (*Gossypium spp.*) By T-Budding Method

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Abstract: Clonal (vegetative) propagation is a technique used to increase productivity and grow large amount of seedlings in a short time from a single plant. The technique is especially useful to protect and increase pure lines. T-budding method used to propagate cotton plant clonally in this study. Experiment was conducted at the experimental fields of East Mediterranean Transitional Zone Agricultural Research Institute in 2010. Split plot design with three replications was used. Twelve meter plot length, 70x40 cm^2 row spacing and suggested cultural practices for the region were applied. T-budding method in cotton was applied in July, August and September. Giza 45 (*Gossypium barbadense* L.) and Fantom (*Gossypium hirsutum* L.) were used as plant materials. Treatments were 1-[Fantom (root stock) + Fantom (scion)], 2-[Giza 45 (root stock) + Giza 45 (scion)], 3-[Giza 45 (root stock) + Fantom (scion)] and 4-[Fantom (root stock) + Giza 45 (scion)]. Graft success rates were significantly different for months and it was found higher within species than between species. The highest graft rates were obtained in July and August. With the application of this method in cotton, it is possible to maintain genotypes for years and propagate them asexually before seed formation using field and greenhouse facilities together. The method is also useful for the improvement of triploid genotypes obtained from breeding programs and important crosses having insufficient seeds.

Keywords: Cotton, T-budding, cloning, *Gossypium hirsutum* L., *Gossypium barbadense* L.

Pamuk (*Gossypium spp.*) Bitkisinin T-Göz Aşısı ile Klonal Üretimi

Öz: Klonal (vejetatif) üretim, bir bitkiden kısa sürede çok sayıda fide üretmek için kullanılan bir tekniktir. Bu teknik, saf hatların muhafazası ve çoğaltılması yönünden kullanışlı bir yöntemdir. Bu çalışmada, pamuk bitkisinin klonal üretimi için T-göz aşısı kullanılmıştır. Çalışma, 2010 yılında, Doğu Akdeniz Geçit Kuşağı Tarımsal Araştırma Enstitüsü'nde yürütülmüş ve bölünmüş parseller deneme deseninde üç tekerrürlü düzenlenmiştir. 70 x 40 cm^2 genişliğinde ekilen bitkiler, bölge koşullarına göre yetiştirilmiştir. Pamukta T-göz aşısının uygulanması Temmuz, Ağustos ve Eylül aylarında yapılmıştır. Giza 45 (*Gossypium barbadense* L.) ve Fantom (*Gossypium hirsutum* L.) çeşitleri bitkisel materyali oluştururken, uygulamalar; 1-[Fantom (anaç) + Fantom (aşı gözü)], 2-[Giza 45 (anaç) + Giza 45 (aşı gözü)], 3-[Giza 45 (anaç) + Fantom (aşı gözü)] ve 4-[Fantom (anaç) + Giza 45 (aşı gözü)] olarak belirlenmiştir. Çalışma sonucunda, aşı tutma oranının aylara göre değiştiği ve en yüksek aşı tutma oranının, Temmuz ve Ağustos ayları ile tür içi uygulamalara ait olduğu saptanmıştır. Ayrıca, bu yöntem ile önemli pamuk genotiplerinin uzun yıllar yaşatılması, çoğaltılması ve korunmasının yanı sıra, ıslah programlarından gelen tohumsuz (triploid) veya yeterli tohuma sahip olmayan melezlerin çoğaltılması yönünden önemli olduğu belirlenmiştir.

Anahtar Kelimeler: Pamuk, T-göz aşısı, klonal üretim, *Gossypium hirsutum* L., *Gossypium barbadense* L.

1. Introduction

Plants are basically having sexual or asexual reproduction systems. Merging male and female gametes to form zygote and thus seed to give new individual known as sexual production. Using vegetative organs such as plant roots, branches and leaves to obtain new individual are called asexual reproduction system. In the sexual reproduction, male and female individuals or organs can be found on different plants or different flowers of the same plant as well as on the same flower depending on the plant species.

Sexual and asexual reproduction has some advantages over each other. Being cheaper and easier seeds obtained by sexual reproduction can be easily stored in small areas. However, some

plants cannot produce seed thus they are not able to grown from seed. With this, some plants can produce seed but it takes long time and effort to have a developed plant. In this case, the asexual reproduction has more advantageous. Insects and wind which are the main factors to pollinate out-crossing plants increase the heterozygosity and deform the varieties by time. In asexual system, in contrast, new individual (clone) has the same genetic constitute with its parent.

Cotton has out-crossing ratio of about 5-10 % but this could be up to 30% depending on the insect population (Mert and Bayraktar 1994; Yolcu 1995; Ünay et al. 1998; Xanthopoloulos and Kechagia 2000; Oğlakçı et al. 2000; Sen et al. 2004; Bozbek et al. 2008). This will cause some segregation problems in the pure cotton cultivars and the uniformity will be disturbed by time. Cotton seed production is not suggested in the regions having high bee population but if it is necessary than selfing need to be done to continue cultivars' pureness. On the other hand, selfing needs intensive use of labor and costs much. Furthermore, Rea (1931) reported that the grafting studies on cotton plants gave successful results.

Grafting with different types (chip budding, scion budding, T-budding) is a method of vegetative production. Today, it especially found application areas in seedling and arboriculture works. It is possible to obtain many plants genetically identical in a short time by using shoots from a single plant and attaching them to other plants by budding. Time saving, continuing breeding works on the triploid plants, saving genotypes susceptible to diseases, continued elite seed production within a year using the field-green house combination, conserving maximum yield and quality of the best plant in an elite level of the commercial cultivar and producing more seed from the wild type plants during the season are some of the advantages of this technique.

The main goal of this work is to search possibility of clonal reproduction of cotton. For this, application of T-budding system and application timing were searched in two different cotton species, *Gossypium hirsutum* L. and *Gossypium barbadense* L.

2. Materials and Methods

2.1. Plant materials

Two different cotton cultivars, Fantom (*Gossypium hirsutum* L.) and Giza 45 (*Gossypium barbadense* L.) were used in the experiment. Giza 45 known as a source of good fiber quality characteristics with stronger to *Verticillium dahlia* Kleb and roots. But has longer vegetation period and lower yield in Turkey conditions. On the other hand, Fantom is an upland cultivar having good yield than Giza 45 cultivar except roots structure. It has cylindrical structure and comparatively less vegetative branches (generally 1-2). First boll opening day of Fantom is about 90 days and micronaire value averages 3.58.

2.2. Field applications

Experiment was conducted at the experimental fields of East Mediterranean Transitional Zone Agricultural Research Institute in 2010 (370 38' N; 36^0 37' E and altitude: 568 m). Plants were sown on May 3, 2010 after 2006 grafting studies in split plot design with 3 replications. Budding applications were used as the main plots while the months were using sub plots. Every plot has got four rows with 12 m length was. Spacing between and within rows were 70 and 40 cm, respectively. Standard cultural practices were applied as suggested by experimental station. Applications were 1-[Fantom (root stock) + Fantom (scion)], 2-[Giza 45 (root stock) + Giza 45 (scion)], 3-[Giza 45 (root stock) + Fantom (scion)] and 4-[Fantom (root stock) + Giza 45 (scion)]. For each application, 20 plants were used as root stock both in replications and in July, August and September. Budding success rate (%) and grafted plant number were inspected regarding the months (July, August and September). To promote branching, the plants used to take scions were cut of the main stem 10-35 cm above the soil surface during June and July. T-bud grafting method was applied as shown in Figure 1.

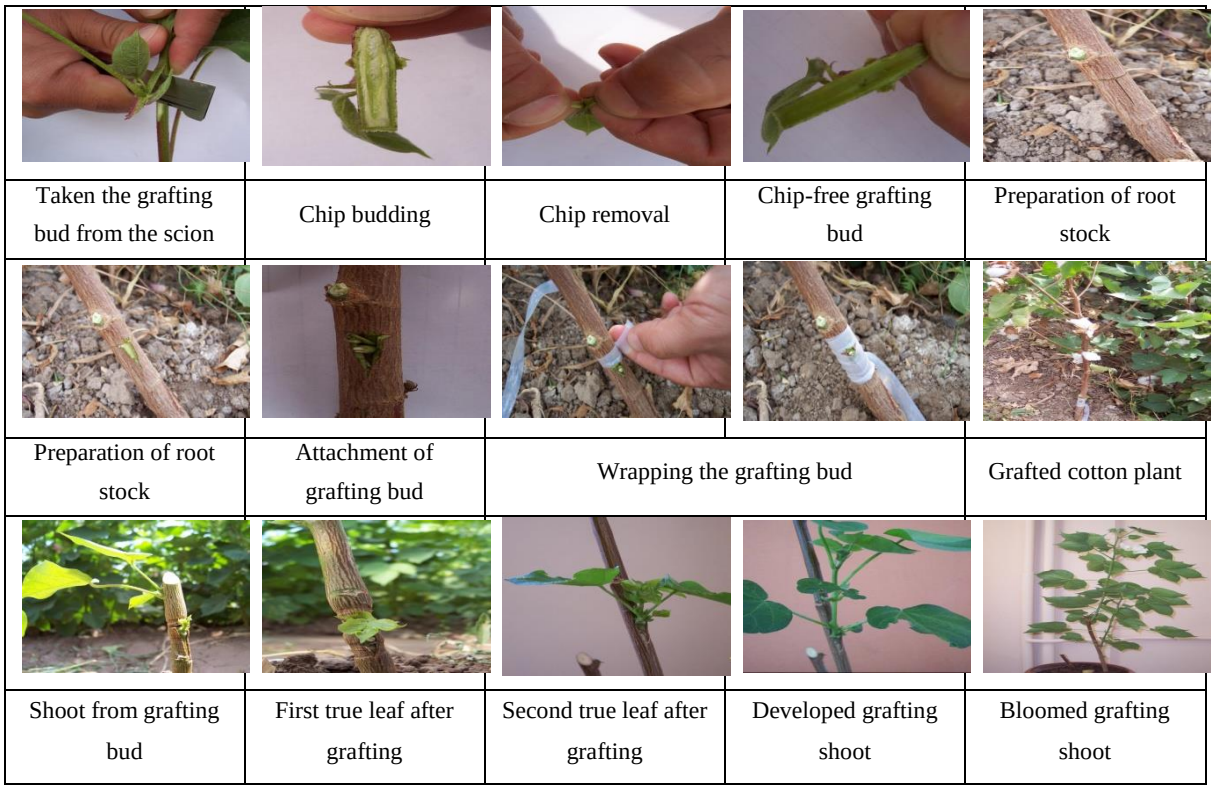

Figure 1. Application of T-budding method followed by shoot development and flowering in cotton
Şekil 1. *Pamukta T-göz aşısı metodunun uygulanması, aşı gözünün sürmesi ve çiçek oluşumu*

Shortly, with the grafting knife cotton plants used as root stock were cut like a T shape on the xylem. Then, grafting bud taken from scion were attached to the opened T-shaped part and wrapped to merge two tissues.

3. Results and Discussion

Variance analysis showed significant differences among the applications (Table 1).

Table 1. Analysis of variance for the grafting applications in cotton
Çizelge 1. *Pamukta T-göz aşısı uygulamasına ait varyans analizi*

Source	Degree of Fredom	Sum of Squares	Mean square	
Treatments	3	41.78	13.93	**
Months	2	66.72	33.36	*
Replication	2	9.72	4.86	
Treatments × Months	6	18.39	3.07	*
Error	22	152.28	6.92	
C. Total	35	288.89		

Results obtained from the experiment were given in tables 2 and 3. Grafted plants were obtained from all the applications. Use of the same cultivars as root stock and scions increased the graft success rate and produced more clones (Table 3).

Table 2. Success of grafting regarding to applications (root stocks + scions) in July, August and September

Çizelge 2. *Uygulamaların Temmuz, Ağustos ve Eylül aylarına ait aşı başarısı*

Applications (Root Stocks + Scions)	Number of Grafted Plants (per 20 plants)			Averages
	July	August	September	
1-[(Fantom + Fantom)]	16.0 a	14.7 ab	14.7 ab	15.1 a
2-[(Giza 45 + Giza 45)]	16.3 a	13.7 abc	12.0 abc	14.0 ab
3-[(Giza 45 + Fantom)]	15.0 ab	13.3 abc	9.7 c	12.7 ab
4-[(Fantom + Giza 45)]	13.7 abc	12.3 abc	11.3 bc	12.4 b
Averages	15.3 a	13.5 ab	11.9 b	13.6
C v (%)				19.4
LSD $_{(0.05)\ Months}$				2.23
LSD $_{(0.05)\ Applications}$				2.57
LSD $_{(0.05)\ Months\ x\ Applications}$				4.46

Average number of grafted plants was the highest (15.1) (75.5 %) in the 1- [Fantom (root stock) + Fantom (scion)] application fallowed by 2-[Giza 45 (root stock) + Giza 45 (scion)] (14.0) (70 %). The lowest number of clones were belong to 4-[Fantom (root stock) + Giza 45 (scion)] (12.4) (62.2 %) and 3-[Giza 45 (root stock) + Fantom (scion)] (12.7) (63.3 %) applications (Tables 2 and 3).

In respect to application time, the highest number of grafted plants (15.3) (76.2 %) was obtained in July application and followed by August (13.5) (67.5%) and September (11.9) (59.6 %) (Table 2).

Table 3. Success ratio of grafting regarding to applications (root stocks + scions) in July, August and September

Çizelge 3. *Uygulamaların Temmuz, Ağustos ve Eylül aylarına ait aşı başarı oranı*

Applications (Root Stocks + Scions)	Graft Success Ratio (%)			Averages (%)
	July	August	September	
1-[(Fantom + Fantom)]	80.0	73.3	73.3	75
2-[(Giza 45 + Giza 45)]	81.7	68.3	60.0	70.0
3-[(Giza 45 + Fantom)]	75.0	66.7	48.3	63.3
4-[(Fantom + Giza45)]	68.3	61.7	56.7	62.2
Averages	76.2	67.5	59.6	67.8

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This is probably caused by climatic change and continued growth of cotton plants. Because of low night temperatures and reaching to harvesting time during September may cause low number of grafted plants.

Although number of grafted plants was lower in between species, successful grafting between species has some advantages (applications 3 and 4). For example, agricultural production can continue in the regions having salinity or disease problem in the soil affecting the plant when tolerant or resistant cultivars selected as root stock. When grafting done at the seedling stage then especially in the small areas infested with *Verticillium wilt*, cotton production can be possible. Another advantage of the grafting is maintaining triploid plants, resulted hybridization

between species having different number of chromosomes, for a long time for further studies without depending on the seed. Cotton can survive for a long time when provided with appropriate conditions and thus using greenhouse will also help breeding efforts continue without breaking. We think that grafting method can also be used in seed production. Thus, single rows of the cultivars are being watched until August or September when the best rows representing the cultivar will be harvested and fiber quality traits determined. As a result of fiber quality measurements, the single best plants from the best rows will be transferred to the plants grown in the greenhouse and many cloned plants obtained from which new clones and seed produced in tracking the time. Since these obtained seed will be the same genetically, when transferred to the field they will be homogenous regarding to yield, quality and phenotype. This one-year study will open new avenues in cotton breeding and provide a tool for the breeders to increase breeding efficiency.

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