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# Transfer of a β-Glucuronidase Marker Gene to Triticale (x*Triticosecale* Wittmack) via Particle Bombardment (Biolistic) Method

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**ABSTRACT**

This study was carried out to investigte the biotechnology laboratory of Department of Field Crops, Ankara University with the aim of investigating the optimum parameters of gene transfer to mature embryo and leaf segment explants of Triticale (x *Triticosecale* Wittmack) via particle bombardment (Biolistic) method. Mature embryo and leaf segment explants obtained from Triticale (x *Triticosecale* Wittmack) were obtained via bombardment with accelerated gold and tungsten particles coated with the plasmid pBI221.23 containing the b-glucuronidase (GUS: u*idA*) marker gene. Different bombardment distances from the stopping plate (6, 9, 12, cm) and different rupture disk pressures (900, 1100, 1550 psi ) were used as physical parameters. Blue spots expressing the b-glucuronidase gene were detected by using a histochemical assay. The variation in the number of blue spots in bombarded explants was used to determine the gene transfer efficiency. The mature embryos of triticale were found to be more amenable for the direct delivery of foreign gene by the particle bombardment technique than the leaf segments. Also the gold particles were more suitable than tungsten particles in the gene transfer efficiency. The highest gene transfer results were obtained in mature embryos when 6 cm bombardment distance and 1100 psi rupture disk pressure were used.

Keywords**:** Particle bombardment; GUS; Marker gene; Gene expression; Triticale; *Triticosecale*

# Partikül Bombardımanı (Biyolistik) Yöntemi ile Tritikaleye (x*Triticosecale* Wittmack) β-Glukuronidaz İşaret Geni Aktarımı

**ESER BİLGİSİ**

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**ÖZET**

Ankara Üniversitesi Ziraat Fakültesi Tarla Bitkileri Bölümü biyoteknoloji laboratuvarında yürütülen bu çalışmanın amacı; Tritikale (x*Triticosecale* Wittmack )'nin olgun embriyo ve yaprak eksplantlarına partikül bombardımanı (Biyolistik)

tekniği ile gen aktarmada kullanılabilecek en uygun parametreleri belirlemektir. Tritikale (x *Triticosecale* Wittmack )'den elde edilen olgun embriyo ve yaprak eksplantları, b-glukuronidaz (GUS: *uidA*) işaret genini içeren pBI221.23 plazmidi ile kaplanmış ve hızlandırılmış altın ve tungsten partikülleri ile bombardıman edilmiştir. Hedef eksplantlara olan farklı bombardıman mesafeleri (6, 9, 12, cm) ve farklı kırılma-diski basınçları (900, 1100, 1550 psi) fiziksel parametreler olarak kullanılmıştır. b-glukuronidaz gen ifadesinin olduğu mavi hücreler histokimyasal bir analiz ile belirlenmiştir. Bombalanan explantlardaki mavi nokta sayılarındaki varyasyon gen transfer etkinliğinin belirlenmesinde ölçüt olarak kullanılmıştır. Tritikale'ye, partikül bombardımanı tekniği ile doğrudan gen aktarımında; olgun embriyoların yapraklara oranla, altının ise tungstene oranla daha iyi olduğu belirlenmiştir. Denemede en iyi sonucu veren olgun embriyolara, bombardıman mesafesinin 6 cm, kırılma-diski basıncının ise 1100 psi olduğu saptanmıştır.

Anahtar Kelimeler: Partikül bombardımanı; GUS; İşaret geni; Gen ifadesi; Tritikale; *Triticosecale*



## 1. Introduction

Triticale is an artificial grain obtained through the hybridization of wheat (*T. durum* Desf. or *T. aestivum* L.) and rye (*S. cereale* L.). These hybrids are made fertile by polyploidy methods and depending on the objectives, spring and winter varieties are utilized.

Considering its interspecific genetic background, triticale which is composed of many species that are resistant to drought becomes more important in recent years due to the present side effects of global warming. Triticale is more often used in feeding of animals when compared to barley, rye and oats however, like many plant species, needs genetic improvement to be able to withstand biotic and abiotic stresses.

Within the last decade the results of an ever increasing number of studies are appearing in the literature regarding biotechnological intervention of triticale. These studies are generally focused on developing *in vito* regeneration methods by utilizing various explant sources of triticale (Padmaja et al 1992; Sirkka & Immonen 1993; Ainsley et al 1998; Vikrant & Rashid 2001; Bohorova et al 2001; Avcı et al 2004). However, molecular breeding studies, specifically, gene transfer studies, are rather limited on triticale. The earliest study on gene transfer to triticale was by Becker et al (1995), and the "bar" marker gene is bombarded to the scutellar tissues obtained from microspores. At the same year Zimny et al (1995) have transferred "*uidA*" and "bar" genes to the scutellar tissues via particle bombardment technique and obtained transgenic plants. However, a later study by Zimny & Lörz, (2000), has revealed the presence of some unstable transformants. Rubio et al (2004) reported the transfer of the "*uidA*" gene to the calli of "Toreto x Presto" hybrid triticale haploids via microprojectile bombardment technique.

In our laboratory, the results of the first gene transfer study were obtained in 1999 by using the particle bombardment technique on wheat (Özgen et al 1999) and the accumulated wisdom throughout the years is decided to be applied to triticale transformation.

Therefore, the objective of this study is to determine the optimum physical and biological conditions required to transform triticale mature embryo and leaf segments by using particle bombardment (biolistic) technique.

## 2. Material and Methods

### *2.1. Plant materials*

In this study; Triticale (x *Triticosecale* Wittmack) cv. "Mikham-2002" hexaploid triticale seeds were used as the source of mature embryos and leaf segments. "Mikham-2002" was especially chosen due to its excellent regeneration capacity (Avcı Birsin and Özgen 2004).

### *2.2. Surface sterilization of triticale mature embryos*

Mature seeds were surface-sterilized in 70% (v $v^{-1}$) ethanol for 5 min, rinsed twice with sterile distilled water, incubated further in commercial bleach for 30 min and rinsed several times in sterile distilled water.

The surface-sterilized seeds were imbibed in sterile distilled water at 33 ºC for 2 h. The mature embryos were aseptically removed with a scalpel and a blade and were placed scutellum upwards(50 embryos), arranged in a circle of 2.5 cm-diameter at the center of the petri plates containing callus induction medium (Murashige and Skoog salts (MS) (1962) supplemented with 20 mg $L^{-1}$ sucrose, 2 mg $L^{-1}$ 2,4-D, 7 mg $L^{-1}$ agar). The dishes were incubated at 25 ºC under darkness for twenty four hours prior to bombardment.

*2.3. Preparation of leaf explants*

Surface sterilized seeds of triticale were grown in jars supplemented with hormone-free MS medium supplemented with 20 mg $L^{-1}$ sucrose. The leaf segment were removed after ten days and placed (14 leaf explants) at the center of the Petri plates.

*2.4. Plasmid vector*

As the plasmid DNA, pBI221.23 (Londsdate et al 1990) containing the *ß- glucuronidase* (GUS) gene and the *hpt* (Hygromycin resistance gene) gene under the control of a dual cauliflower mosaic virus (CaMV) '35S' promoter (Figure 1) was used. All bombardments were carried out with the Bio-Rad Biolistic® PDS 1000 / He particle delivery system according to the manufacturer's instructions.

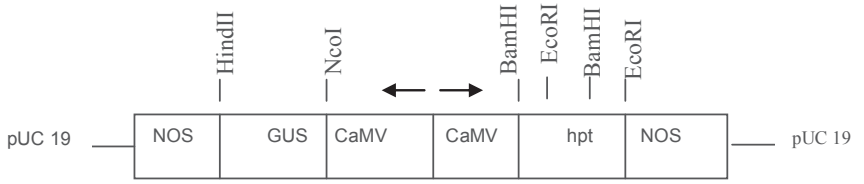


**Figure 1- The map of plasmid pBI221.23**

*Şekil 1- Bombardımanda kullanılan pBI221.23 plazmidinin şematik görünümü*

*2.5. Preparation of microprojectiles*

All bombard-ments are carried out according to the principles of Sanford et al (1987) and modifications developed by Özgen et al (1999) and Önde et al (2001) respectively. Gold or Tungsten macroprojectiles (60 mg $ml^{-1}$) were washed in absolute ethanol once and resuspended in sterile 50% (v $v^{-1}$) glycerol. For every six bombardments 50 µl gold or tungsten suspension aliquots were placed in fresh eppendorf tubes and 5 µl plasmid DNA (1 µg $µl^{-1}$ ), 50 µl of 2.5 M $CaCl_2$ and 20 µl of 0.1 M spermidine were added. After centrifugation at 10000 rpm for 10 seconds the pellet was resuspended in 60 µl of absolute ethanol.

*2.6. Preparation of macroprojectiles*

Each macro-projectile is loaded with 6 µl of suspension and allowed to dry under vacuum. The explants were bombarded at a rupture disk pressure-stopper disk distance (psi-cm) of 900-6, 900-9, 900-12, 1100-6, 1100-9, 1100-12, 1550-6, 1550-9 and 1550-12 with three replicates.

*2.7. Assay of gene expression*

Transient expression of the GUS gene was detected by the "histochemical staining" method (Jefferson, 1987) which utilizes the chromogenic substrate 'X-gluc' (5-bromo-4-chloro-3-indolyl β-D- glucuronic acid).

All the bombarded explants were incubated for 48 hours at 26 ºC under darkness. At the end of this post-bombardment incubation all explants are transferred to individual tubes for GUS assay. The assay solution (Jefferson, 1987) was added and the tubes are incubated at 37 ºC under darkness for at least 6 hours up to 24 hours.

*2.8. Evaluation of data*

Study results were evaluated based on the number of blue spots (Ritala et al 1994). As gene transfer could only occur in embryos placed in X-Gluc solution and blue spotted leaves, these embryos and leaves were investigated under microscope and the numbers of blue spots were recorded. Results

obtained for each one of 50 embryos and 14 leaves in bombarded petri; the number and percentage values of blue spotted embryos and leaves, total number of blue spots and blue spot number per embryo and leaf were calculated. The success of bombardment was estimated as the number of blue spotted embryos and leaves and the resulting percentage values of blue spotted embryos and leaves. Obtained data from three replications were analyzed according to "Factorial ANOVA" in MSTAT-C statistical software, and the difference between means was controlled by Duncan test. Percentage data were transformed to arcsine before analysis. Chi-square independence test was used to determine the difference between bombardment pressures and distances (Steele & Torrie 1960).

## 3. Results and Discussion

In this study a total of 1350 embryos were bombarded with gold particles (1.6 µm in average diameter) and a total of 8243 blue spots on 538 embryos (Figure 2) were identified which are indicative of transient β-GUS gene expressing groups of cells. With this respect, 39.6% of the bombarded embryos displayed at least one transient gene expression event (Table 1). For leaf explants, a total of 378 explants were bombarded with gold particles and a total of 6994 blue spots on 348 leaf explants (Figure 3) were counted. For leaf explants, 92% of the bombarded material yielded at least one transient gene expression event (Table 3).

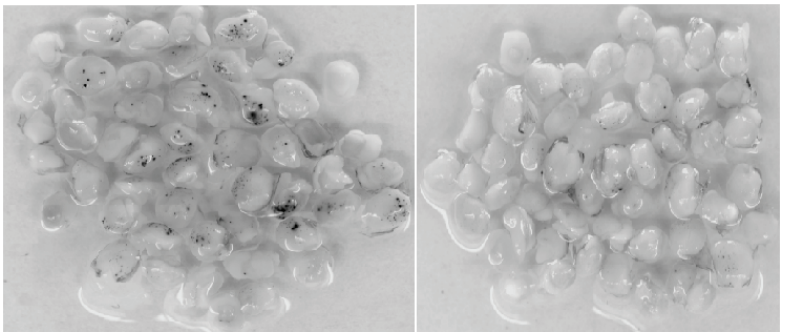

**Figure 2- Transient GUS expression in mature t*riticale* embryos bombarded with gold (left) and tungsten (right) particles, assayed 48 h after bombardment (1100 psi / 6 cm and 1100 psi / 9 cm, respectively)**

*Şekil 2- Olgun tritikale embriyolarında bombardımandan 48 saat sonra GUS görünümü (sağda tungsten partikülleri ile 1100psi / 9 cm; solda altın partikülleri ile 1100psi / 6cm)*

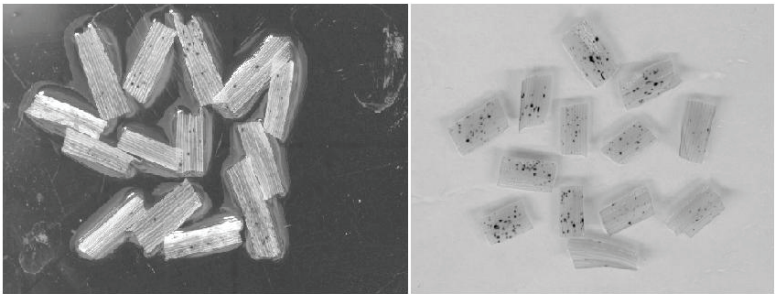

**Figure 3- Transient GUS expression in *Triticale* leaf explants bombarded with gold (left) and tungsten (right) particles, assayed 48 h after bombardment (1550 psi / 12 cm and 1550 psi / 9 cm, respectively)**

*Şekil 3- Tritikale yaprak eksplantlarında bombardımandan 48 saat sonra GUS görünümü (sağda tungsten partikülleri ile 1550 psi/ 9 cm; solda altın partikülleri ile 1550 psi / 12cm)*

For comparing the efficiency of gene transfer, tungsten particles were also used. For this purpose, a total of 1350 embryos were bombarded with tungsten particles (M10: 0.7 µm in average diameter) and a total of 6900 blue spots were counted on 626 embryos. In this respect 46.7% of bombarded embryos yielded at least one transient gene expression event. For leaf explants, a total of 378 explants were bombarded with tungsten particles and a total of 3405 blue spots on 319 leaf explants were counted. For leaf explants, 84.3% of the bombarded material yielded at least one transient gene expression event (Table 3).

**Table 1- Blue spot numbers obtained in bombardments made on mature embryos of triticale with tungsten and gold particles using three different pressures and distances**

*Çizelge 1- Olgun tritilale embriyolarında üç farklı basınç ve uzaklıkta altın ve tungesten partiküllari ile yapılan bombardımanda elde edilen mavi nokta sayıları*

| *Particles* | *Pressure (psi)* | *Distance (cm)* | *The number of embryos with blue spots* | *The ratio of embryos with blue spots (%)* | *The number of blue pots* | *Blue spot / Embryo* | |
|---|---|---|---|---|---|---|---|
| | | | | | | *Total embryos* | *Embryos with blue spots* |
| Gold | 900 | 6 | 15.0 ±2.57gh* | 30.0 ±2.30hı | 122.3± 2.60ıj | 2.4 ±0.51fg | 9.1 ±3.23fgh |
| | | 9 | 16.0 ±1.73gh | 32.0 ±3.46ghı | 202.3 ±7.50gh | 3.3± 0.38efg | 10.5 ±0.04efg |
| | | 12 | 11.6± 0.33h | 23.3 ±0.66ı | 152.0 ±3.40hı | 3.0 ±0.41efg | 12.9± 1.48c-f |
| | 1100 | 6 | 30.3 ±2.60abc | 60.6 ±5.20abc | 581.0 ±17.89a | 11.6 ±0.93a | 18.2 ±0.51a |
| | | 9 | 16.0 ±0.57gh | 32.0 ±1.15ghı | 292.0 ±2.30e | 5.8± 0.05d | 17.7 ±2.61ab |
| | | 12 | 15.0 ±0.57gh | 30.0± 1.14hı | 185. 0 ±8.66gh | 3.7 ±1.09ef | 12.6 ±0.19def |
| | 1550 | 6 | 26.0 ±2.30bcd | 53.0 ±2.64cde | 364.0 ±4.61d | 7.28 ±0.09c | 14.0 ±0.09b-e |
| | | 9 | 26.0 ±1.73bcd | 52.3 ±2.02cde | 450.3 ±18.76c | 8.9 ±0.23b | 16.9± 1.11abc |
| | | 12 | 23.3± 2.02def | 46.6 ±4.05ef | 432.0 ±3.46c | 8.6 ±0.18bc | 12.8±1.27def |
| Tungsten | 900 | 6 | 25.0 ±2.19cde | 50.0 ±4.16de | 208.3 ±2.02fg | 4.1 ±0.02e | 8.0 ±0.56gh |
| | | 9 | 20.3 ±1.36efg | 40.6 ±2.34fg | 125.0 ±2.30ıj | 2.5 ±0.62fg | 5.9 ±0.13hı |
| | | 12 | 16.0± 0.81gh | 32.0 ±0.81ghı | 92.0± 8.08j | 1.8 ±0.16g | 5.7 ±0.50hı |
| | 1100 | 6 | 18.3 ±1.19fg | 36.6 ±1.92gh | 226.0 ±11.5f | 4.5 ±0.23de | 12.5± 0.73def |
| | | 9 | 34.3 ±2.32a | 68.6 ±4.43a | 535.0 ±33.48b | 10.7±0.09a | 15.0 ±0.74a-d |
| | | 12 | 18.0 ±1.22fg | 36.0 ±1.15gh | 191.3 ±3.1fgh | 3.8 ±0.06ef | 10.9 ±1.35d-g |
| | 1550 | 6 | 28.3± 1.59bcd | 56.6 ±1.76bcd | 392.0 ±14.4d | 7.8 ±0.86bc | 13.3 ±1.37c-f |
| | | 9 | 31.0 ±1.47ab | 65.3 ±2.40ab | 429.0 ±5.77c | 8.5 ±0.69bc | 13.8 ±0.34b-e |
| | | 12 | 17.3 ±0.87g | 34.6 ±0.66gh | 104.6 ±7.28j | 2.0 ±0.14g | 5.8 ±0.52ı |
| Gold | | | 19.9b | 39.6b | 305.2a | 6.1a | 13.9a |
| Tungsten | | | 23.2a | 46.7a | 255.5b | 5.1b | 9.8b |
| | 900 | | 17.3c | 34.1c | 144.7c | 2.8b | 8.7c |
| | 1100 | | 22.0b | 44.0b | 335.1b | 6.7a | 14.5a |
| | 1550 | | 25.3a | 51.4a | 361.4a | 7.2a | 12.3b |
| | | 6 | 23.8a | 47.2a | 315.6b | 6.3a | 12.5a |
| | | 9 | 23.9a | 48.5a | 333.4a | 6.7a | 13.4a |
| | | 12 | 16.8b | 33.7b | 192.3c | 3.8b | 9.7b |
| *P value* | | | | | | | |
| Particles(A) | | | 0.0003 | 0.0000 | 0.0000 | 0.0002 | 0.0000 |
| Pressure(B) | | | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Distance (C) | | | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| AxBxC | | | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0179 |

*, Means followed by the same letter are not significantly different at P= 0.05

According to results of variance analysis performed on data of bombardments made on mature embryos of triticale using two different particles and three different pressure and distance values, interactions of particle, pressure, distance and particle x pressure x distance were found statistically significant. Duncan test results analyzing this difference are given in Table 1. As can be seen in Table, tungsten particles(23.2) were observed to create higher number of blue spots on mature embryos compared to gold particle (19.9). Gold particles formed blue spots on approximately 19.9 out of 50 embryos, while the total number of blue spots was 305.2 and the number per embryo was 13.9; on the other hand, tungsten particles created blue spots on around 23.2 out of 50 embryos, while the total number of blue spots was 255.5 and the number per embryo was 9.8. The best bombardment result performed on mature embryos of triticale with gold particles was obtained with 1100psi pressure and 6cm distance (the mean number of blue spotted embryos was 30.3 and the number of blue spots per embryo was 18.2); on the other hand, the best result of tungsten particles was obtained with 1100psi pressure and 9cm distance (total number of blue spotted embryos was 34.3 and the blue spot number per embryo was 15) (Table 1).

For comparing the effects of microprojectile type (gold vs. tungsten) on the *ß- glucuronidase* transient gene expression level in embryogenic cells, a Chi-Square analysis was performed (Table 2). The results clearly indicated the superiority of gold particles in 1100psi-6cm combination for mature embryo bombardments ($\chi^2$ = 10.58**) whereas tungsten particles became superior in 1100psi–9cm combination ($\chi^2$ = 25.32**) when compared with gold particles.

**Table 2- Comparative of gold and tungsten particles in terms of transient GUS expression in mature *triticale* embryos**

*Çizelge 2- Olgun Tritikale embriyolarına altın ve tungsten partikülleri ile GUS aktarımının karşılaştırılması*

| *Pressure-distance Psi-cm* | *The ratio of embryo with blue spots (%) gold* | *The ratio of embryo with blue spots (%) tungsten* | *Ki-Kare* |
|---|---|---|---|
| 900-6 | 30.0 | 50.0 | 7.50** |
| 900-9 | 32.0 | 40.6 | 1.22 |
| 900-12 | 23.3 | 32.0 | 1.48 |
| 1100-6 | 60.6 | 36.6 | 10.58** |
| 1100-9 | 32.0 | 68.6 | 25.32** |
| 1100-12 | 30.0 | 36.0 | 0.564 |
| 1550-6 | 53.0 | 56.6 | 0.134 |
| 1550-9 | 52.3 | 65.3 | 2.97 |
| 1550-12 | 46.6 | 34.6 | 2.508 |

**, $P \leq 0.01$

According to the variance analysis results of bombardment performed on leaf explants of triticale with gold and tungsten particles using three different pressure and distance values, interaction of particle, pressure and particle x pressure x distance were found statistically significant. The results of Duncan test results analyzing this difference are given in Table 3. As can be seen in Table, gold particles created higher mean number of blue spots (12.8) on leaf explants compared to tungsten particles (11.8). Gold particles formed blue spots on 12.8 out of 14 leaf explants in petri, while the total number of blue spots was 259 and the blue cspot number per leaf explants was 19.6; on the other hand, tungsten particles created blue spots on 11.8 out of 14 leaf explants, while the total number of blue spotted leaf explants was 126.1 and the number of blue spots per leaf explants was 10.3. The best result of gold particle bombardment performed on leaf explants of triticale was obtained with 1100 and 1550psi pressure and 12cm distance (the mean number of blue spotted leaf explants was 13.3 and 14 and the blue spot number per explants was 24.6 and 23.9 respectively). As for tungsten particles, the best result was obtained with 1550psi

pressure and 9cm distance (number of total blue spotted explants was 13.3 and the blue spot number per explants was 16.9) (Table 3).

For comparing the effects of microprojectile type (gold vs. tungsten) on the *ß- glucuronidase* transient gene expression level in leaf cells, a Chi-Square analysis was performed (Table 4). Within the low pressure groups (900-12, 1100-6, 1100-9 and 1100-12) superiority of gold particles are evident ($\chi^2$ = 25.642**, 5.534*, 8.404**, and 8.404** respectively) however at 1550-9 combination Tungsten particles differed significantly when compared with gold particles ($\chi^2$ = 8.404**).

**Table 3- Blue spot numbers obtained in bombardments made on leaf explants of triticale with tungsten and gold particles using three different pressures and distances**

*Çizelge 3- Tritikalenin yaprak eksplantlarına üç farklı basınç ve uzaklıkta altın ve tungsten partiküllari ile yapılan bombardımanda elde edilen mavi nokta sayıları*

| *Particles* | *Pressure (psi)* | *Distance (cm)* | *The number of leaf explant with blue spots* | *The ratio of leaf explants with blue spots (%)* | *The number of blue pots* | *Blue spot / Leaf explant* | |
|---|---|---|---|---|---|---|---|
| | | | | | | *Total leaf explants* | *Leaf explants with blue spots* |
| Gold | 900 | 6 | 13.0 ±0.00ab* | 92.8 ±0.00abc | 189.0 ±4.13d | 13.4± 2.46de | 14.5 ±1.19cd |
| | | 9 | 13.0± 0.00ab | 92.8 ±0.00abc | 266.3 ±8.87b | 19.0 ±0.74bc | 20.4 ±0.60b |
| | | 12 | 13.0 ±0.57ab | 92.8 ±4.12abc | 268.3± 13.72b | 19.1 ±0.98bc | 20.5 ±0.45b |
| | 1100 | 6 | 13.6 ±0.33ab | 97.6 ±2.40a | 282.6 ±5.54b | 20.1 ±0.63b | 20.7 ±1.04b |
| | | 9 | 13.3 ±0.33ab | 95.2 ±2.40ab | 313.6 ±4.33a | 22.4 ±0.89ab | 23.4 ±1.74ab |
| | | 12 | 13.3 ±0.33ab | 95.2 ±2.40ab | 329.0 ±17.03a | 23.4 ±1.66a | 24.6 ±0.79a |
| | 1550 | 6 | 11.3± 0.66b | 80.9 ±4.76c | 161.6± 1.76e | 11.5± 0.12ef | 14.3± 1.05cd |
| | | 9 | 11.3 ±0.88b | 80.9 ±6.26c | 184.6 ±2.90de | 13.1 ±1.51de | 17.1 ±1.94c |
| | | 12 | 14.0±0.00 a | 100.0± 0.00a | 336.0 ±5.85a | 23.9 ±1.47a | 23.9 ±1.47a |
| Tungsten | 900 | 6 | 11.6 ±1.2ab | 83.3 ±3.45bc | 113.0 ±2.51f | 8.0± 1.01gh | 10.5 ±0.52e |
| | | 9 | 12.3± 0.33ab | 88.0 ±2.36abc | 69.3 ±1.72g | 4.9 ±0.38hı | 5.5 ±0.61f |
| | | 12 | 8.6 ±0.88c | 61.8 ±3.49d | 36.3± 1.59h | 2.5 ±0.48ı | 4.0 ±0.10f |
| | 1100 | 6 | 12.3 ±0.88ab | 88.0 ±1.26abc | 188.6± 5.74d | 13.4 ±0.41de | 15.2 ±0.54cd |
| | | 9 | 11.3 ±1.2b | 80.9 ±6.59c | 112.3 ±3.68f | 8.0 ±1.02gh | 10.4 ±0.35e |
| | | 12 | 11.3 ±0.88b | 80.9± 6.29c | 101.3 ±1.96f | 7.2 ±0.57gh | 8.9 ±0.17e |
| | 1550 | 6 | 12.3± 0.88ab | 88.0 ±1.26abc | 170.6± 9.12de | 12.1± 1.06e | 13.4± 1.29d |
| | | 9 | 13.3 ±0.33ab | 95.2 ±2.4ab | 223.3 ±10.88c | 15.9 ±0.93cd | 16.9 ±1.19c |
| | | 12 | 13.0 ±0.57ab | 92.8 ±4.12abc | 120.0 ±11.57f | 8.5± 0.82fg | 9.2 ±0.77e |
| Gold | | | 12.8a | 92.0a | 259.0a | 18.5a | 19.6a |
| Tungsten | | | 11.8b | 84.3b | 126.1b | 9.0b | 10.3b |
| | 900 | | 11.9a | 85.3b | 157.1c | 11.2c | 12.5c |
| | 1100 | | 12.5a | 89.6a | 221.3a | 15.8a | 17.2a |
| | 1550 | | 12.5a | 89.7a | 199.4b | 14.2b | 15.6b |
| | | 6 | 12.3a | 88.5a | 184.3b | 13.1a | 14.7a |
| | | 9 | 12.4a | 88.8a | 194,9a | 13.9a | 15.4a |
| | | 12 | 12.2a | 87.2a | 198.5a | 14.2a | 15.2a |
| *P value* | | | | | | | |
| Particles(A) | | | 0.0020 | 0.0001 | 0.0000 | 0.0000 | 0.0000 |
| Pressure(B) | | | 0.2175 | 0.0664 | 0.0000 | 0.0000 | 0.0000 |
| Distance (C) | | | - | - | 0.0181 | 0.2604 | - |
| AxBxC | | | 0.3900 | 0.1240 | 0.0000 | 0.0000 | 0.0007 |

*, Means followed by the same letter are not significantly different at P = 0.05

**Table 4- Comparative of gold and tungsten particles in terms of transient GUS expression in *Triticale* leaf explants**

*Çizelge 4- Tritikale yaprak eksplantlarına altın ve tungsten partikülleri ile GUS aktarımının karşılaştırılması*

| *Pressure-distance Psi-cm* | *The ratio of leaf explant with blue spots (%) (gold)* | *The ratio of leaf explant with blue spots (%) (tungsten)* | *Ki-Kare* |
|---|---|---|---|
| 900-6 | 92.8 | 83.3 | 3.432 |
| 900-9 | 92.8 | 88.0 | 0.83 |
| 900-12 | 92.8 | 61.8 | 25.642** |
| 1100-6 | 97.6 | 88.0 | 5.534* |
| 1100-9 | 95.2 | 80.9 | 8.404** |
| 1100-12 | 95.2 | 80.9 | 8.404** |
| 1550-6 | 80.9 | 88.0 | 1.416 |
| 1550-9 | 80.9 | 95.2 | 8.404** |
| 1550-12 | 100.0 | 92.8 | 2.866 |

**, $P \leq 0.01$

The success of microprojectile bombardment technique relies on physical, chemical and biological factors. The explant source is the principal biological factor affecting the success of this technique. In our study we chose mature embryos and leaf segment of triticale as the targets for microprojectile bombardment due to their easy manipulation and availability throughout the whole year. These explant materials are also frequently used as an alternative source of totipotent target cells for microprojectile bombardment-mediated transformation in other cereals such as; mature embryos of oat (Torbert et al 1998), rice (Valdez et al 1998), wheat ( Özgen et al 1999; Patnaik et al 2006) and leaf segment of wheat (Rajyalakshmi et al 1991).

When gold particles are used, 92 % of all leaf explants displayed at least one blue spot whereas for mature embryos this was 39.6 %. With this respect, the leaf explants are superior over the mature embryos. This result might be attributed to the even and continuous surface structure of leaves, where majority of the particles that reached the petri plates have penetrated cells. Mature embryos not only have uneven surfaces, but also the spaces occurred between individual embryos during target preparation might causes some particles to strike at these spaces and hence causing reduced percentage we have observed. The same profile is observed when tungsten particles are used which was 84.3% for leaf explants whereas 46.7% for mature embryos, respectively.

However, a contradiction became evident when the numbers of β-Glucuronidase gene expressing cells (total number of blue spots) are counted. With this respect mature embryos are found to be superior over the leaf explants regardless of microprojectile type. This might be explained by the texture of the explants. Since the cells of the mature embryos are softer and devoid of waxy layers, more particles penetrated these cells and resulted with higher numbers of β-Glucuronidase gene expressing cells (total number of blue spots) when gold ( 8243 vs 6994) and tungsten (6900 vs 3405) are used.

Being one of the physical factors, the type of the microprojectile also affects the efficiency of the transformation. At the beginning of the microprojectile bombardment research majority of the researchers preferred to use tungsten particles due to their low cost and ease of availability. However, the problems related with the DNA coating and their phytotoxic nature has led the researchers to develop alternative sources of microprojectiles. One such alternative is found out to be the gold particles. Although expensive, the gold particles are neither phytotoxic nor they have problems with the DNA-coating process. We have tested both of these particles. Regardless of explant type, the highest numbers of blue spots were observed when gold particles are used (8243 vs 6900 for mature embryos,

6994 vs 3405 for leaf explants) whereas tungsten particles performed poorly especially when leaf explants are used. One of the reasons for this poor performance of tungsten particles might be attributed to the differences between the average particle size content of tungsten (0.7 µm) and gold (1.6 µm). As a general rule, the smaller the diameter of the particles the slower velocities they gain through their journey and thus the penetration to the cells become problematic. In addition to this problem, "the finer" nature of the particles also causes "finer expression events" which sometimes makes the visualization of the GUS expression events very difficult ( Rasco-Gaunt et al 1999). Two published reports by a Polish research group (Krysiak et al 1999a,b) indicated the negative effects of tungsten microprojectiles on the integrity of DNA. Similar to our results, three independent groups ( Charest et al 1993; Ratnayaka and Oard, 1995; Mohri et al 2000) indicated the superiority of gold particles over tungsten particles in microprojectile bombardment process.

## 4. Conclusions

To the best of our knowledge, this study is the first to demonstrate optimal parameters needed to transfer genes in to matured embryos and leaf explants of triticale (x *Triticosecale* Wittmack) via particle bombardment technique. Our results clearly demonstrated the superiority of mature embryos over the leaf explants in terms of overall gene expression events (number of blue spots) as well as the superiority of gold particles over the tungsten particles. We have also established the optimal rupture disk pressure-stopper disk distance (psi-cm) combination for matured embryos as1100–6 (gold) and 1100–9 (tungsten) whereas for leaf explants 1550–12 (gold) and 1550–9 (tungsten).

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