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Callus Induction and Plant Regeneration from Mature Embryos of Oat (*Avena sativa* L.)

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Abstract: The aim of this study, which was conducted in Ankara University, Faculty of Agriculture, Department of Field Crops, biotechnology laboratory in 1998-1999, was to determine the callus induction and plant regeneration capacity of oat (*Avena sativa* L.). The mature embryos of 10 oat genotypes were utilised for callus induction. The statistical analysis of the results revealed that callus induction and plant regeneration capacity were dependent on the oat genotype. However, a significant relationship was not established between callus induction and regeneration capacity and, furthermore, the number of regenerated plants was determined to be directly related to the callus production capacity.

Key Words: *Avena sativa* L., plant regeneration, embryo culture, callus induction

Yulafta (*Avena sativa* L.) Olgun Embriyolardan Kallus Oluşumu ve Bitki Rejenerasyonu

Özet: Ankara Üniversitesi Ziraat Fakültesi Tarla Bitkileri Bölümü biyoteknoloji laboratuvarında 1998-1999 yıllarında yapılan bu araştırmanın amacı; yulafta kallus oluşumu ve rejenerasyon yeteneğini belirlemektir. On yulaf çeşit ya da hattının kullanıldığı araştırmada; kallus oluşturmada olgun embriyolardan yararlanılmıştır. Elde edilen veriler istatistik olarak değerlendirildiğinde; kallus oluşumu ve rejenerasyon kapasitesinin çeşit ve hatlara göre değiştiği, kallus oluşumu ve rejenerasyon kapasitesi arasında önemli bir ilişkinin olmadığı; rejenere bitki sayısının doğrudan kallus oluşumuna bağlı olduğu saptanmıştır.

Anahtar Sözcükler: *Avena sativa* L., bitki rejenerasyonu, embriyo kültürü, kallus oluşumu

Introduction

The utilisation of biotechnology in plant breeding is largely dependent on callus induction and subsequent plant regeneration from various explant sources. The success in this process is affected predominantly by genotypes and the type of explant material (1,2). The earliest tissue culture studies in the oat (*Avena sativa* L.) were initiated in the 1960s by using mature embryos as the explant source (3). For achieving oat callus cultures, various tissues including immature embryos (3,4,5), young leaves (6), apical meristems (7,8), mesocotyl tissues (9), root tips (10) and pollen (11,12) have been employed.

Results similar to the best callus induction were achieved when immature embryos were used as an explant source in oats (3). However, immature embryos being only available at a very limited time in a growing season, generally necessitates growth of oat plants in greenhouses or in climate-controlled rooms (2,13), which not only increases the labour constraints but also hampers experimental flexibility. Although mature embryos produce lower callus yields, their availability throughout the year makes them an excellent explant source.

The aim of this study, therefore, was to determine the callus induction and plant regeneration potential of various oat genotypes by using mature embryos as the explant source.

Materials and Methods

In this study, 10 oat genotypes (Ankara-76, Ankara – 84, A- 803, A- 804, A- 805, A- 821, A- 822, A- 823, A- 824 and A- 825) were used in Ankara University, Faculty of Agriculture, Department of Field Crops, biotechnology laboratory during 1998-1999.

The floral bracts of the aforementioned genotypes were removed from the mature seeds. These seeds were surface sterilised in 70% ethanol for 5 min, rinsed twice with sterilised distilled water, incubated further in 50% sodium hypochlorite solution for 25 min, and rinsed 7 times in sterilised distilled water. The surface sterilised seeds (ca. 80 seeds per genotype) were incubated at 33ºC for 2 h in sterilised distilled water for imbibition. The mature embryos were removed from the imbibed seeds and placed, scutellum-up, on MS medium (14) supplemented with 20 mg/l sucrose, 2 mg/l 2,4-D, 7 mg/l agar and incubated at 25 ± 1ºC for 14 days in darkness. At the end of this incubation period, the callus tissues were transferred to hormone-free MS medium for initiating root and shoot development (regeneration) and incubated for 5 weeks at 25 ± 1ºC in a 16 h light (2000 lux) 8 h dark photoperiod.

The 1-2 cm tall plantlets that developed roots and shoots were transferred to magenta boxes containing hormone-free MS medium and incubated for a further month. As the roots of these plantlets reached 10-12 cm, they were transferred to pots containing garden soil. In order to attain high humidity (acclimatisation) the pots were covered with plastic film and kept at 25 ± 1ºC in a 16 h light 8 h dark photoperiod. After 3 weeks of maintenance, the plants were vernalised at 4ºC for 2 weeks and subsequently transferred to soil in a greenhouse.

For each genotype a completely randomised design with 4 replications (20 embryos per replication) was constructed to collect data on the number of embryos inducing callus, callus weight, number of regenerating calli and the number of plants growing in soil. The data were subjected to analysis of variance and least significant difference tests. Correlation coefficients between the tested parameters were also calculated (15).

Results and discussion

Callus Induction

The callus induction from mature embryos of oat became visible within the third day of culturing, although this was noted to be dependent on the genotype. However, the formed calli were nodular and white to cream in colour regardless of genotype. On the fourteenth day of culturing, the average diameter of the calli was measured to be 3-4 mm (Figure 1). The data on callus initiation and plant regeneration obtained from oat genotypes is given in Table 1. The callus induction was found to show a variation within genotypes; while line A-821 was displaying 95% callus induction and 84% of these callus were regenerated, line A-805 displayed the least callus induction (50%), from which only 75.8% were regenerated (Table 1).

In plant tissue cultures, a desirable genotype is expected to possess high callus induction and plant regeneration capacity. However, numerous studies have shown the absence of such a relationship between callus induction and plant regeneration capacity and, thus, the independence of these characters from each other (2,16,17). Our data also confirmed this finding; while in line A-804 the callus induction was 66.3% and the regeneration capacity was 91.4%, these ratios were observed to be 72.5% and 69.3% respectively for the Ankara 76 variety (Table 1). Furthermore, the absence of a statistically significant relationship between callus induction and plant regeneration can be clearly seen in Table 2.

The average weight of the induced callus within the experiment was calculated to be 1.1 g. The highest callus weight was measured in line A-821 (1.4 g), which was also noted to be displaying the highest callus induction potential (95%). This result suggested a positive relationship between callus induction and callus weight. Furthermore, a statistically significant correlation analysis result between the callus induction and callus weight (Table 2) also supported this hypothesis.

A statistically significant positive correlation between callus induction and culture efficiency ($r= 0.915$ **) was noted, as shown in Table 2. This result implies that an increase in callus induction in genotypes will accompany an increase in culture efficiency (Table 1). Furthermore, statistically significant positive correlations between callus weight and culture efficiency and number of plants transferred to soil ($r= 0.729$** and $r = 0.564$ **, respectively) were also noted (Table 2). Our data suggested that genotypes with high callus induction also caused an increase in the number of plants transferred to soil.

Table 1. Average values of 10 oat genotypes on callus induction and plant regeneration.

Genotypes	Callus Induction (%)	Callus Weight (g)	Regeneration Capacity[1] (%)	Culture Efficiency[2] (%)	Number of plants transferred to soil
Ankara-76	72.5 bc[3]	1.1ab	69.3 c	50.0 bc	4.8bc
Ankara-84	77.5 ab	1.0 ab	88.5 ab	68.8 ab	4.3bc
A-803	82.5 ab	1.1ab	91.8 a	76.3 a	8.0ab
A-804	66.3 bc	0.9 b	91.4 a	61.3 abc	1.5c
A-805	50.0 c	0.8 b	75.8 bc	38.8 c	6.0ab
A-821	95.0 a	1.4 a	84.0 abc	80.0 a	9.3a
A-822	72.5 bc	1.0 b	85.1 ab	62.5 abc	5.5ab
A-823	71.5 bc	0.9 b	88.3 ab	62.5 abc	7.0ab
A-824	80.0 ab	1.1 ab	93.0 a	75.0 a	5.5ab
A-825	85.0 ab	1.2 ab	89.1 ab	76.3 a	6.8ab
Total		10.5			58.7
Average	75.3± 3.8	1.1±0.1	85.6±2.4	65.2±4.1	5.9±0.7

[1] number of regenerated callus / number of embryos inducing callus x 100
[2] number of regenerated callus / number of cultured embryos x 100
[3] The average values assigned the same letter are statistically insignificant.

Table 2. The correlation coefficients of 10 oat genotypes on callus cultures derived from mature embryos.

Characters	Correlation coefficients between characters				
	1	2	3	4	5
1 Callus Induction (%)	-	0.870**	0.335	0.915**	0.644**
2 Callus Weight (g)	-	-	0.118	0.729**	0.564**
3 Regeneration Capacity (%)	-	-	-	0.678**	0.147
4 Culture Efficiency (%)	-	-	-	-	0.551**
5 Number of plants transferred to soil	-	-	-	-	-

**Significantly different at P = 0.01

Plant Regeneration

The induced callus structures when transferred to regeneration medium started to form green spots rapidly. In this hormone-free medium, the callus structures yielded numerous leaves and roots (Figure 2). Approximately 2 months after callus induction, the average number of plants that were transferred to soil varied between 1.5 and 9.3, depending on the genotypes, and an average total of 58.7 plants were grown to maturity in the greenhouse (Table 1, Figure 3).

While the average regeneration capacity of oat genotypes were 85.6%, line A-824 was observed to display the highest (93%) and Ankara 76 variety was observed to display the lowest (69.3%) regeneration capacity (Table 1). These results, which demonstrated the effects of oat genotypes on plant regeneration, were also in line with previous studies (3,18).

Although some genotypes were found to possess high callus induction potential and high plant regeneration capacity, as can be seen in Table 2, a statistically significant relationship was not detected between regeneration capacity and callus induction and the number of plants transferred to soil. While the effect of regeneration capacity of callus on the number of regenerated plants was found to be absent (r=0.147), the number of regenerated plants were observed to be determined by callus induction (r= 0.644 **), as seen in Table 2. This hypothesis is further supported by the absence of a statistically significant relationship between callus

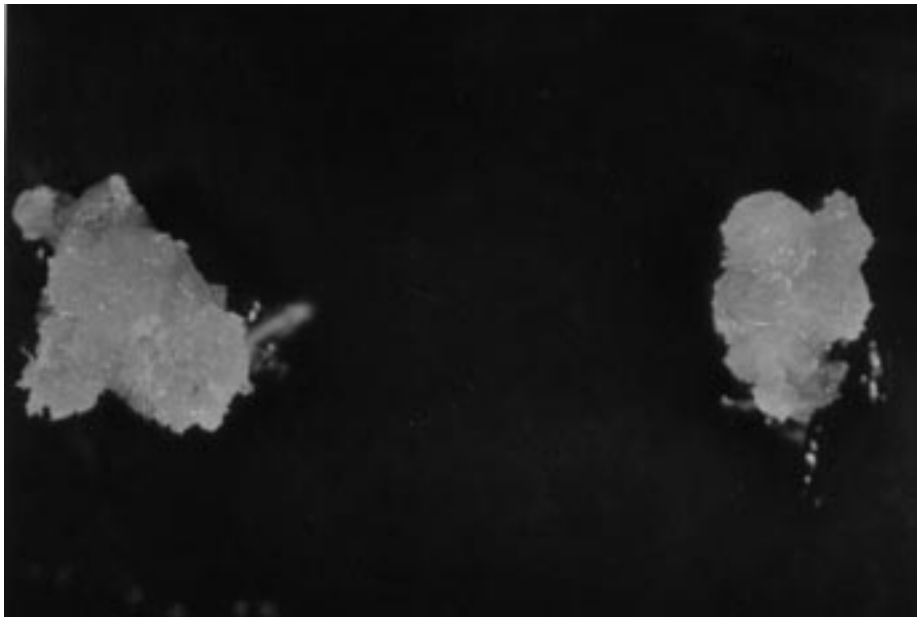

Figure 1. Mature embryo-derived callus structures of oat on day 14 of culturing.

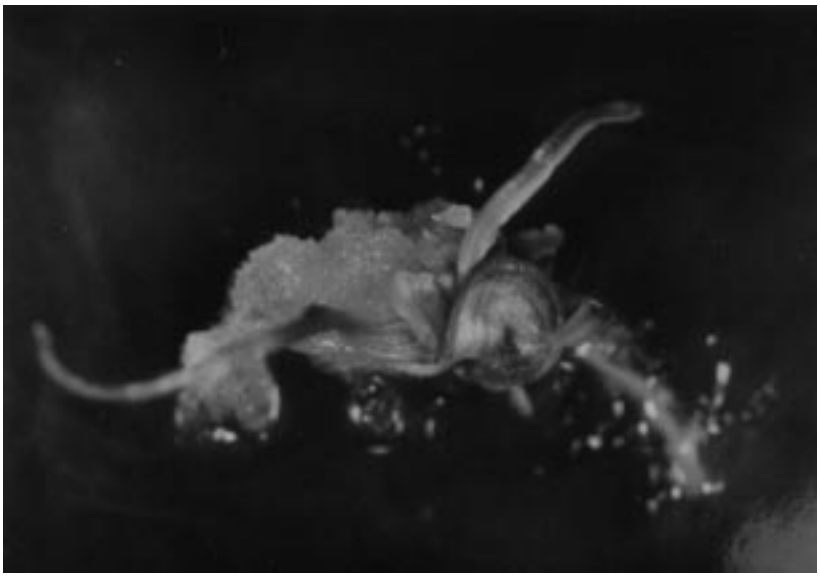

Figure 2. Regenerating callus on hormone-free MS medium.

Figure 3. Regenerated plants that were transferred to soil.

induction and regeneration capacity (r= 0.335). As a consequence, for increasing the number of regenerated plants that will be transferred to soil, utilisation of genotypes with high callus induction capacity is proposed to be a necessity.

References

1. Özgen, M., Türet, M., Özcan, S., Sancak, C. Callus induction and plant regeneration from immature and mature embryos of winter durum wheat genotypes. Plant Breed. 115: 455-458, 1996.
2. Özgen, M., Türet, M., Altınok, S., Sancak, C. Efficient callus induction and plant regeneration from mature embryo culture of winter wheat (*Triticum aestivum* L.) genotypes. Plant Cell Reports. 18: 331-335, 1998.
3. Rines, H.W., McCoy, T.J. Tissue culture initiation and plant regeneration in hexaploid species of oats. Crop Sci. 21:837-842, 1981.
4. Bregitzer, P., Somers, D.A., Rines, H.W. Development and characterisation of friable, embryogenic oat callus. Crop Sci. 29: 789-803, 1989.
5. Bregitzer, P., Bushnell, W.R., Rines, H. W., Somers, D. A. Callus formation and plant regeneration from somatic embryos of oat (*Avena sativa* L). Plant Cell Reports 10: 243-246, 1991.
6. Chen, H., Xu, G., Loschke, D. C., Tomaska, L., Rolfe, B. G. Efficient callus formation and plant regeneration from leaves of oats (*Avena sativa* L.). Plant Cell Reports 14: 393-397, 1995.
7. Zhang, S., Zhang, H., Sticklen, H. B. Production of multiple shoots from shoot apical meristems of oat (*Avena sativa* L). Journal of Plant Physiology, 148:6, 667-671, 1996.
8. Bommineni, V. R., Walden, D. B. Shoot apical meristem culture of maize, wheat, barley and oat. Maize genetics Cooperation News Letter. 64: 78-79, 1990.
9. Chen, Z. H., Zhuge, Q.G., Sundqvist, C. Oat leaf base: tissue with an efficient regeneration capacity. Plant Cell Reports. 14:6, 354-358, 1995.
10. Chen, Z., Klockare, R., Sundqvist, C. Origin of somatic embryogenesis is proliferating root primordial in seed derived oat callus. Hereditas-Landskrona. 120:3, 211-216, 1994.
11. Kiviharju, E., Puolimatka, M., Pehu, E. Regeneration of anther-derived plants of *Avena sterilis*. Plant Cell, Tissue and Organ Culture 48: 147-152, 1997.
12. Rines, H. W. Oat anther culture: genotype effects on callus initiation and the production of a haploid plant. Crop Sci. 23: 268-272, 1983.
13. Torbert, K. A., Rines, H. W., Somers, D. A. Transformation of oat using mature embryo-derived tissue cultures. Crop sci. 38: 226-231, 1998.
14. Murashige, T., Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473-497, 1962.
15. Düzgüneş, O., Kesici, T., Kavuncu, O., Gürbüz, F. Araştırma ve Deneme Metodları (İstatistik Metodları-II). A.Ü.Z.F. Yayınları: 1021, Ders Kitabı:295, Ankara, 1987.
16. Sears, R.G., Deckard, E.L. Tissue culture variability in wheat: callus induction and plant regeneration. Crop Sci. 22: 546-550, 1982.

17. Chowdhury, S. H., Kato, K., Yamamato, Y., Hayashi, K. Varietal variation in plant regeneration capacity from immature embryo among common wheat cultivars. Jpn. J. Breed. 41: 442-450, 1991.

18. Rines, H.W., McCoy, T.J. Effect of genotypes on initiation of tissue cultures from embryos and anthers of oats (*Avena* spp.). Am. Soc. Agron. Abst. p. 67, 1980.