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NATURE OF GENE ACTION FOR IMMATURE EMBRYO CULTURE ABILITY IN WHEAT (*Triticum aestivum L*)

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ABSTRACT

The objective of this study was to determine the genetic parameters involved in immature embryo culture ability in wheat (*Triticum aestivum L*). For this purpose, five lines and two cultivated varieties were used. These lines were: C.B243, C.B245, C.B249, C.B251 and C.B253. While, the two testers were Gemmieza 9 and Gemmieza 10. The results showed the presence of highly significant differences among genotypes for all *in vitro* traits. This indicated that embryogenic calli induction and its regeneration were affected by the genotypes of the immature embryo donor plants. The line C.B 251 was the most responsive for both embryogenic calli ratio and regenerable calli ratio, while the line C.B243 was the best combiner among other lines in the case of callus fresh weight. The beast combination for all traits was the cross C.B 251× Gemmieza 9. Furthermore, the results revealed that both additive and non-additive genetic variances played an important role in the inheritance of immature embryo culture traits. However, the magnitude of non- additive genetic variance was larger than their corresponding estimates of additive genetic variances for callus fresh weight and regenerable calli ratio. This finding was emphasized by the dominance degree value which exceeded unity with respect to these traits. Therefore, it could be concluded that using F1 hybrids for immature embryo culture improvement the ability to induce high ratio of plantlets.

Keywords: *Triticum aestivum* - gene action - heritability – immature embryo culture

INTRODUCTION

Wheat is one of the most important cereal crops used in human food and animal feed in Egypt and all over the world. For many years ago, several investigators have directed their research to increase the productivity of wheat per unit area by either introducing more yielding varieties or improving agricultural practices.

Plant regeneration from cultured cells and tissues is required for successful application of biotechnology in modern plant breeding programs. Plant regeneration of many cultivars of wheat obtained from culture of immature embryos has been established by Shah *et al.* 2003, Nasircilar *et al.* 2006 and Yasmin *et al.* 2009. In this connection, several factors affect *in vitro* cell culture. It was found that the most important factor is genetic structure of explants. *In vitro*, the response of wheat tissues and the efficiency of callus induction have been recognized for callus growth rate and plant regeneration which were found to be genotype dependent as reported by Zhou and Lee 1983; Racz *et al.* 1993; Ozgen *et al.* 1998; Yadav *et al.* 2000; Anapiiaeva 2000; Schween and Schwenkel 2003; Yadav and Chawla 2001; El-Sherbeny *et al.*, 2001. In this respect, De Buyser *et al.* (1992) detected the involvement of few genes in controlling plant regeneration. Heritability values ranged from 0.6 and 0.7 for callus formation and plant regeneration indicating the importance of additive effects as observed by Lszar *et al.* (1984). In addition

Milach *et al.* (1991b) concluded that the plant regeneration in wheat was controlled by both additive and dominance effects, with reciprocal effects having no importance. Lange *et al.* (1995) studied plant regeneration, embryogenesis, early germination and organogenesis of Brazilian wheat genotypes and concluded that the additive and dominance effects were significant for all studied traits. The dominance effects, especially for plant regeneration were elevated, and epistatic effects were also expressive. Barakat (1996) estimated the gene actions for five *in vitro* traits of immature wheat (*Triticum aestivum L.*) and found that the contribution of additive gene effects was not as important as the dominance gene effects. Abd El-Maksoud (2003) determined the genetic control of immature embryo culture ability and found that both additive and non-additive genetic variance contribute to the genetic expression of immature embryo culture ability traits. On the other hand, Abdel-Hady (2006) found that additive genetic effects were dominant and played the major role in the inheritance of callus growth from immature embryo culture of wheat.

Therefore, this investigation aimed to determine the importance and magnitudes of the different genetic parameters involved the inheritance of the in immature embryo culture ability using line by tester mating design.

MATERIALS AND METHODS

This investigation was carried out in tissue culture Lab., Genetics department, Faculty of Agriculture, Mansura University during the years 2009 to 2012.

Plant Materials:

The plant materials used in this study included five wheat (*Triticum aestivum L*) lines and two cultivated varieties uses as testers. These lines were: C.B243, C.B245, C.B249, C.B251 and C.B253. While, the cultivated varieties were Gemmieza 9 and Gemmieza 10. The grain of these plant materials were pervaded by Gemmieza Research Station, ARC, Egypt. The origin, pedigree and symbol of these lines and varieties are shown in Table1. During the winter season of 2009/2010, these five lines and two varieties were sown at the Experimental farm of Gemmieza Research Station, ARC, Egypt. At flowering time, the five lines were pollinated by the two check varieties according to 5 lines x 2 tester fashion in order to produce ten top crosses. During the winter season of 2010/2011 all crosses i.e. C.B243/Gemmieza 9, C.B243/Gemmieza 10, C.B245/Gemmieza 9, C.B245/Gemmieza 10, C.B249/Gemmieza 9, C.B249/Gemmieza 10, C.B251/Gemmieza 9, C.B251/Gemmieza 10, C.B253/Gemmieza 9 and C.B253/Gemmieza 10 were sown at Faculty of Agriculture Experimental Station, Mansoura University for immature culture purpose under field condition.

Table 1: The origin, pedigree and symbol of the plant materials

No	Pedigree	Origin
C .B243	Shi#4414/Crow"S" SWM11508-1AP-1AP-5AP-1AP-4AP-0AP	Mexico
C .B245	Laj2965	Syria
C .B249	Hd2206/Hork"s" CM39808-58M-2Y-4M-1Y-1M-1Y-0B	Mexico
C .B251	Cno/Mfd/Mon"s"/3/Gov/Az//Mus"s" CM72602-02AP-300AP-3AP-2AP-300L-0AP	Mexico
C .B253	47777*2//Fkn/Gb/3/Nac#5/4/Buc"S"/Pvn"S" CM66684-B-1M-6Y-2M-2Y-1M-0Y	Mexico
Gemmieza 9	Ald"s"/Hmac//Cmh74A-630/SxCGm4583-5Gm-1Gm-0Gm	Egypt
Gemmieza 10	Maya74"s"/on//1160147/3/BB/GLL/4/CHAt"s"/5/ Crow"s"CGm5820-3Gm-1Gm-2Gm-0Gm	Egypt

Embryo culture procedure

To initiate callus, young spikes were collected about 15 days post anthesis from each genotype. Immature caryopses were surface sterilized under sterile conditions by immersing them for one min in 79% ethanol followed by immersion in 0.1% mercuric chloride solution with 2 drops of Tween 20 as a wetting agent for 20 mints and rinsed for 7 times in sterile double distilled water. Immature embryos were excised from each grain aseptically and cultured with the scutellm side up on the Murashige and Skoog (1962) containing 20% sucrose and supplemented with 2.0 mg/L 2,4-dichlorophenoxy acetic acid (2,4-D) as recommended by Abd El-Maksoud (2003). The pH was adjusted to 5.8 before autoclaving at 121°C for 20 minutes. The *in vitro* experimental design was a randomized complete blocks with 10 crosses in three replications. Each Petri dish, containing 10 embryos was considered as one experimental unit. After 28 days of incubation at 25C°±2C°in darkness the total number of calli and the fresh weight of calli were recorded. Calli were transferred to MS medium with 0.5 NAA mg/L and 0.5 mg/L kinetin as growth regulator. Cultures were incubated at 22C°±2C°, under 16 hour illumination fluorescent light for 30 days. Then, the regenerable calli, which have green shoot primordial (green spot) were counted and transferred to the same regeneration medium in the same condition.

The data were recorded on the embryonic calli ratio (number of calli per responding embryo), fresh weight of initiated calli derived from each embryo and regenerable calli ratio (number of calli that produced green shoot primordial per number of transferred embryogenic calli).

Statical analysis:

In order to normalize the distribution of the percentage data which fall between 0.0 to 1.00 were transformed by using arcsine $x^{1/2}$ function prior to statistical analysis. The data were analyzed by using the ordinary analysis of variance method in order to test the significances of the differences among the ten crosses resulted from 5 x 2 lines by tester mating design. If the crosses mean square were found to be significant, there was a need to proceed for further analysis, i.e. line by tester analysis as indicated by Steel and Torri 1960 and Kemphorne 1957.

RESULTES AND DISCUSSION

The analysis of variance and the mean squares of the crosses for *in vitro* studied traits are presented in Table 2. Highly significant differences were found among crosses for embryonic calli ratio and regenerable calli ratio. While, the magnitudes of the mean square were significant in the case of callus fresh weight. This indicated that embryogenic calli induction and its regeneration are affected by the genotype of immature embryo donor and the further analysis for partition this genetic variance to its components could be valid.

Table 2: Mean squares of Line x Tester analysis of variance for *in vitro* traits

S.O.V	d.f	Embryogenic calli ratio	Callus fresh weight	Regenerable calli ratio
Replication	2	0.68	3.31	1.21
Crosses (C)	9	2.11**	11.88**	15.31**
Lines (L)	4	3.56	10.13	12.22
Testers (T)	1	0.363	3.07	8.59
L × T	4	1.09	15.83*	20.07**
Error	18	0.52	4.57	1.64

*, ** significant at 0.05 and 0.01 level of probability, respectively.

In order to partitioning the crosses to its components, Line x Tester analysis of variance was made for studied *in vitro* traits and the results in table 2 showed that the lines and testers were not significant. This indicated that there were no diversity existed among lines and testers in ability to respond to immature embryo culture. On the other hand, lines x tester mean squares were only significant for callus fresh weight and highly significant for regenerable calli ratio. Their mean squares were larger in magnitude than the corresponding values of the mean squares for lines and testers. This finding indicated the importance of SCA than GCA in the genetic behavior of these genotypes with respect to these *in vitro* traits.

Mean performance of crosses for the studied traits are presented in Table 3. The greatest mean frequency for all *in vitro* traits was observed in the combination C.B 251× Gemmieza 9 with means of: 5.87, 22.28 and 10.51 for embryogenic calli ratio, callus fresh weight and regenerable calli ratio, respectively. The means showed that no specific cross was inferior for all studied traits. However, C.B 253×Gemmieza 9 was the inferior cross for embryogenic calli ratio, the C.B 249× Gemmieza 10 was the inferior for callus fresh weight and the C.B 249× Gemmieza 9 was the lowest cross in regenerable calli ratio.

Table 3: Mean performance of crosses for *in vitro* studied traits

Crosses	Embryogenic calli ratio	Callus fresh weight	Regenerable calli ratio
C.B243× Gemmieza 9	4.50	19.33	3.66
C.B243× Gemmieza 10	4.30	20.7	4.41
C.B245× Gemmieza 9	4.40	19.79	4.53
C.B245× Gemmieza 10	4.37	17.38	4.28
C.B249× Gemmieza 9	3.10	16.9	2.73
C.B249× Gemmieza 10	3.43	16.54	3.74
C.B251× Gemmieza 9	5.87	22.28	10.51
C.B251× Gemmieza 10	4.20	17.21	2.95
C.B253× Gemmieza 9	3.07	16.98	2.92
C.B53× Gemmieza 10	3.53	20.26	3.63
LSD 5%	1.022	3.028	1.81
LSD 1%	1.50	4.46	2.67

Estimates of general combining ability effects of lines and testers for studied *in vitro* traits are given in Table 4. Positive and negative estimates would indicate that a given much better or much poorer than the average of the group involved with it in line x tester mating design. Among the lines examined, C.B 251 appeared to be the best one for embryogenic calli ratio and regenerable calli ratio. While C.B243 was the best combiner among lines in the case of callus fresh weight. For testers, the variety Gemmieza 9 was better than Gemmieza 10 which showed positive largest magnitudes for all *in vitro* traits. Thus, these good combiner varieties possess favorable gene for improving hybrids and could be utilized in a traditional breeding program for improving the ability to embryo culture.

The specific combining ability effects of the crosses for the studied traits are presented in Table 5. It could be seen from this Table that the best specific combination was C.B 251× Gemmieza 9 which exhibited highest positive value for improving all *in vitro* traits. This finding indicated that the best combination must be resulted from crossing two good general combiners.

Table 4: General combining ability effects for lines and testers for all studied *in vitro* traits

Lines	Embryogenic calli ratio	Callus fresh weight	Regenerable calli ratio
C.B243	0.32	1.28	-0.30
C.B245	0.31	-0.15	0.07
C.B249	-0.81*	-2.02*	-1.10
C.B251	0.96*	1.01	2.39**
C.B253	-0.87*	-0.12	-1.06
SE	0.29	0.87	0.52
Tester			
Gemmieza9	0.11	0.32	0.54
Gemmieza10	-0.11	-0.32	-0.54
S.E	0.186	0.55	0.33

*, ** significant at 0.05 and 0.01 level of probability, respectively.

Table 5: Specific combining ability effects for line x tester crosses for all studied *in vitro* traits

Crosses	Embryogenic calli ratio	Callus fresh weight	Regenerable calli ratio
C .B 243× Gemmieza 9	-0.01	-1.00	-0.91
C .B 243× Gemmieza 10	0.01	1.00	0.91
C .B 245× Gemmieza 9	-0.09	0.88	-0.41
C .B 245× Gemmieza 10	0.09	-0.88	0.41
C .B 249× Gemmieza 9	-0.28	-0.14	-1.04
C .B 249× Gemmieza 10	0.28	0.14	1.04
C .B 251× Gemmieza 9	0.72	2.20	3.24**
C .B 251× Gemmieza 10	-0.72	-2.20	-3.24**
C .B 253× Gemmieza 9	-0.34	-1.96	-0.89
2 C .B 53× Gemmieza 10	0.34	1.96	0.89
S.E	0.42	1.23	0.74

** significant at 0.01 level of probability, respectively.

The estimation of the additive (σ^2A) and non-additive (σ^2D) genetic variance in addition to heritability in brood (h^2_b) and narrow sense (h^2_n) as well as dominance degree ratio (D.d) were presented in Table 6. The negative values obtained for variances were considered equal to zero during the calculations of heritability and dominance degree. The results indicated that the magnitudes of non- additive genetic variances were larger than their corresponding estimates of additive genetic variances
for callus fresh weight and regenerable calli ratio, indicating the predominance genes with non-additive effects in the inheritance of these traits. On the other hand, the magnitude of additive genetic variance was only larger than non- additive genetic variance for embryogenic calli ratio. This suggested that both additive and non- additive genetic variance may contribute to the genetic expression of immature embryo culture traits. These results are supported by other findings by Barakat (1994) and Abd El-Maksoud (2003).

High broad- sense heritability estimates and low narrow sense estimates were recorded for all *in vitro* studied traits. These values ranged from 45.08 to 78.96 for callus fresh weight and regenerable calli ratio, respectively. This result indicated that non-additive genes play the major role of genetic effects in the expression of these traits. In addition, the dominance degree ratio was larger than unity in the cases of callus fresh weight and regenerable calli ratio, emphasizing the role of non-additive genetic variance in the genetic expression of these traits. These results are in common agreement with Lange *et al.* (1997), who found that a complex gene action with additive and dominant effects would be controlling *in vitro* traits.

Table 6: Genetic parameters for all studied *in vitro* traits

	Embryogenic calli ratio	Callus fresh weight	Regenerable calli ratio
σ^2A	0.412	-1.80	-2.07
σ^2D	0.190	3.75	6.14
h^2_b %	53.59	45.08	78.96
h^2_n %	36.69	0.00	0.00
D.d	0.68	>1	>1

REFERENCES

Abd El-Maksoud, M. M. (2003). Heterosis and genetic control of immature embryo culture ability in Egyptian wheat (*Triticum aestivum L.*) Egypt. J. Pant Breed.7 (1): 363-373.

Abdel-Hady, A. H. (2006). Heterosis and combining ability effects for callus growth of wheat (*Triticum durum, desf*). *In vitro*. J. Appli. Sci. Res. 2 (6): 360-363.

Annapiiaeva BB (2000). The effect of genotype on the rate of regeneration of plants in a microspore culture of *Triticum aestivum L.* Genetika 36: 505-509.

Barakat, M. N. (1994). Combining abilities of *in vitro* traits in wheat *(Triticum aestivum L.)* immature embryo cultures. Euphtica 79(3): 169-175.

Barakat, M. N. (1996). Estimation of genetic parameters for *in vitro* traits in wheat immature embryo cultures involving high X low regeneration capacity genotypes. Euphatica 87 (2): 119-125

De Buyser, J., Marcotte, J.L. and Henry, Y. (1992). Genetic analysis of *in vitro* wheat somatic embryogenesis. Euphytica 63: 265-270.

El-Sherbeny, G.A.R., S. Sato, S.M. Al-Otay K, T. Clement and P.S. Baenziger (2000). Effect of genotype and 2,4-D concentration on callus induction from immature embryo of new Egyptian wheat cultivars (*Triticum aestivum L.*). J. Agric Sci. Mansoura Univ. 25 (12): 7677 – 7683.

Kempthorne, O. (1957). An introduction to genetic statistics. New York: John Wiley Sons, London: Chapman and Hall. Ltd.

Lange, C.E.,Federizzi, L.C., Carvalho, F.I.F., Dornelles, A.L.C. and Handel,C.L. (1997). Genetics of *in vitro* organogenesis and precocious germination of wheat embryos.*Theor. Appl. Genet. 51*: 81-86.

Lange, C.E., Federizzi, L.C., Carvalho, F.I.F., Tavares, M.J.C.S. and Dornelles, A.L.C. (1995).Genetic analysis of somatic embryogenesis and plant regeneration of wheat (*Triticum aestivum* L.). *J. Genet. & Breed. 49*: 195-200.

Lszar, M.D., baenziger, P.S. and Schaeffer, G.W. (1984).Combining abilities and heritability of callus formation and plantlet regeneration in wheat (*Triticum aestivum L*) anther cultures. Theor. Appl. Genet. 68: 131-134.

Milach, S.C.K, Federizzi, L.C., Carvalho, F.I, F. and Barbosa Neto, J.F. (1991). Variabilidadegeneticapara a regeneracao de plantas no cultivo de calos de trigo.Pesq.Agrop.Bras. 26: 1969- 1974

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Nasircilar, A.G., Turgut, K. and Fiskin, K. (2006). Callus induction and plant regeneration from mature embryos of different wheat genotypes. Pak. J. Bot. 38 (2): 637-645.

Ozgen M, Turet M, Altiinok S and Sancak C (1998). Efficient callus induction and plant regeneration from mature embryo culture of winter wheat (*Triticum aestivum L.*) genotypes. Plant Cell Rep 18: 331-335.

Racz I, Raldi E, Lalztity D, Buzas B and Aczel M (1993). Callus cultures and plant regenration from mature embryos in winter wheat. Acta Agronomica Hungarica 42: 255-260.

Schween G and Schwnkel HG (2003). Effect of genotype on callus induction, shoot regeneration and phenotypic stability of regenerated and phenotypic stability of regenerated plants in the greenhouse of *Primula spp.* Plant Cell Tissue Organ Cult 72: 53-61.

Shah, M.I, Jabeen, M. and Ilahi, I. (2003). *In-vitro* callus induction, its proliferation and regeneration in seed explant of wheat (*Triticum aestivum L.*). var. LU-26s. Pak. J. Bot. 35(2): 209-217

Steel, R. G. and Torrie, J. H. (1960). Principles and procedures of statistics. Mc-Graw Hill Book Company, INC. New York.

Yadav MK, Singh NK and Garg GK (2000). Development of lines of Indian wheat genotypes for efficient regeneration using mature embryos. In: Symposium on Biotechnology for sustainable agriculture, 27-29 April 2000, G.B. Pant University of Ag & Tech, Pantnagar, India.

Yadav R and Chawla HS (2001). Interaction of genotype, growth regulators and amino acids on plant regeneration from different developmental states of influorescence in wheat. J Genet Breeding 55: 261-267.

Yasmin, S., Khan, I.A. Khatri, A., Seema, N., Nizamani, G.S. and Arain, M.A. (2009). *In vitro* plant regeneration in bread wheat (*Triticum aestivum L.*). Pak. J. Bot. 41 (6): 2869-2876.

Zhou MD and Lee TT (1983). Selectivity of auxin for induction and growth of callus from excised embryos of spring and winter wheat. Canadian J Bot 62: 1393-1397.

طبيعة الفعل الجينى فى قابلية الأجنة غير الناضجة لزراعة الأنسجة فى القمح

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أجريت هذه الدراسة بهدف تحديد الفعل الجينى المتحكم فى قابلية الأجنة غير الناضجة لزراعة الأنسجة وقد تم استخدام خمسة سلالات كأمهات هى C.B243, C.B245,C.B249, C.B251 and C.B253 وصنفان كاباء هما جميزة ٩ وجميزة ١٠ وتم التهجين بينهما بنظام السلالة x الكشاف.

وقد أظهرت اختبارات المعنوية أن هناك اختلافا عالى المعنوية بين التراكيب الوراثية لجميع الصفات المعملية مما يدل على أن قابلية الأجنة غير الناضجة لاستحداث كالوس وتكوين نباتات خضراء يتأثر بالخلفية الوراثية للمصدر النباتى للأجنة.

كانت أكثر السلالات استجابة لزيادة معدل الكالوس الجنينى و تكوين نباتات خضراء هى: C.B 251 بينما كانت السلالة C.B243 الاقصل فى وزن الكالوس فى حين كان الهجين C.B 251× Gemmieza 9 افضل الهجن استجابة لجميع الصفات المدروسة. بالاضافة لذلك اشارت النتائج الى ان توريث هذه الصفات يتحكم فيها كل من الفعل الجينى المضيف والفعل الجينى غير المضيف (السيادى) والتى كانت أعلى من الفعل الجينى المضيف بالنسبة لصفتى وزن الكالوس والقدرة على انتاج نباتات خضراء ولذلك كانت تقديرات معامل السيادة تزيد عن الواحد الصحيح.

وعموما فان نتائج هذه التجربة تلقى الضوء على امكانية تحسين قابلية الأجنة غير الناضجة لزراعة الأنسجة فى القمح من خلال استخدام نباتات الجيل الأول.

قام بتحكيم البحث

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