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Inheritance of somatic embryogenesis and organ regeneration from immature embryo cultures of winter wheat *

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Summary. Diallel analyses of F_1 and reciprocal crosses among five winter wheat lines show that additive, non-additive, and cytoplasmic genetic effects were significant in the genetic control of somatic embryogenesis, shoot, and root induction frequencies as well as in numbers of somatic embryos, shoots, and roots. However, additive genetic effect appears to be most important since, in most cases a larger portion of the cross variation was accounted for by general combining ability. The results suggest that somatic embryogenesis and organ regeneration in winter wheat can be improved through genetic manipulation. Due to the presence of maternal effects, it may be critical to use a suitable genotype as a female parent in a selection program.

Key words: Wheat – Diallel analysis – Somatic embryogenesis – Plant regeneration – In vitro culture

Introduction

The application of cellular and molecular biology to the improvement of wheat depends upon the ability to initiate, manipulate, and finally regenerate plants from in vitro cultures. Performance in culture of major crops has been shown to be influenced by medium components (Sears and Deckard 1982; Datta and Wenzel 1987), genotype (Maddock et al. 1983: Magousson and Bornman 1985; Mathias and Simpson 1986), and other environments (Lazar et al. 1984). Tissue culture response has been shown to be under genetic control in several cereal crop species such as maize (Beckert and Qing 1984; Tomes and Smith 1985; Hodges et al. 1986), barley (Hanzel et al. 1985; Caligari et al. 1987) and wheat (Bullock and Baenziger 1982; Lazer et al. 1984; Mathias and Fukui 1986).

We have conducted factorial experiments to assess the effects of genotype, 2,4-D concentration, and light/dark conditions on somatic embryogenesis and organ regeneration of winter wheat immature embryos in culture. Significant genotypic effects were found for somatic embryogenesis induction and organ regeneration frequencies. This study was designed to investigate the genetic control of somatic embryogenesis and organ regeneration in immature embryo culture of winter wheat.

Materials and methods

Plant material

Diallel crosses including F_1s and reciprocals were made among five hexaploid hard red winter wheat cultivars adapted to the U. S. Southern Great Plains: 'Arkan', 'Mit', 'Sturdy', 'Tam 101', and 'Vona'. All parental plants were grown in a controlled greenhouse where day/night temperatures were approximately 25 °C/15 °C during flowering. Hand emasculation techniques were employed and immature embryos were collected from caryopses of these hybrids 14–17 days after pollination. Immature seeds were surface-sterilized by immersion in 70% ethanol (v/v) for 1.5 min, then immersion in 10% chlorox (v/v) with 7 drops of detergent per 100 ml for 5 min, and finally rinsing four times with sterile distilled water. Embryos were isolated from the sterilized immature seeds and placed on assigned media with the shoot coleorhiza axis in contact with the medium.

Medium and culture methods

The basic culture medium was that of Murashige and Skoog (1962) (MS medium), supplemented with 150 mg L-asparagine, 20 g sucrose, 2 g Gelrite, and 0.75 g $MgCl_2$ per liter, and two concentrations of 2,4-D (2,4-dichlorophenoxyacetic acid) unless

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Table 1. Mean squares from diallel analysis of variance. Mean squares for embryogenesis, shoot, and root induction frequencies (EIF, SIF, and RIF, respectively) were estimated from transformed values (arcsin $\sqrt{y}$)

Source	df	EIF	SIF	RIF	EN	SN	RN
1.0 mg/l 2,4-D							
Crosses	19	599.57**	856.85**	485.31**	17.71**	10.50**	3.77**
GCA	4	2415.32**	3481.21**	1411.80**	28.88**	38.67**	9.21**
SCA	5	153.01**	196.41**	185.66**	12.42**	4.45**	2.22*
Reciprocal	10	96.55**	137.32**	264.53**	15.88**	2.64**	2.38**
Maternal	4	115.37**	159.00**	346.83**	19.03**	4.19**	3.63**
Error	60	14.92	20.94	58.95	2.48	0.74	0.74
CV, %		10.25	11.52	11.04	21.34	17.37	9.85
0.5 mg/l 2,4-D							
Crosses	19	614.47**	758.75**	479.81**	22.81**	13.46**	5.11**
GCA	4	2352.49**	2724.52**	1218.23**	49.16**	39.11**	9.71**
SCA	5	130.84*	130.95**	163.83	14.56**	3.39**	1.06
Reciprocal	10	161.09**	286.34**	342.44**	16.39**	8.24**	5.29**
Maternal	4	219.25**	404.84**	160.61	23.13**	17.33**	6.97**
Error	60	43.06	39.24	79.36	3.75	0.75	0.68
CV, %		21.97	19.62	11.71	34.35	23.15	8.93

*,** Significant at the 5% and 1% levels of probability, respectively

Table 2. Mean squares from combined diallel analysis of variance. Mean squares for embryogenesis, shoot, and root induction frequencies (EIF, SIF, and RIF, respectively) were estimated from transformed values (arcsin $\sqrt{y}$)

Source	df	EIF	SIF	RIF	EN	SN	RN
2,4-D (D)	1	2436.10**	2437.11**	1703.29**	121.45**	58.32**	12.05**
Crosses (C)	19	1127.68**	1529.11**	840.65**	36.98**	21.80**	7.56**
GCA	4	4711.14**	6097.38**	2544.07**	72.70**	75.73**	18.37**
SCA	5	150.63**	275.22**	322.10**	23.69**	7.09**	1.66*
Reciprocal (R)	10	182.82**	328.75**	418.56**	29.34**	7.59**	6.20**
Maternal	4	216.77**	403.81**	401.39**	41.99**	16.68**	9.11**
C × D	19	86.36**	86.48**	124.47*	3.53	2.16**	1.32*
GCA × D	4	56.66	108.35**	85.95	5.34	2.06*	0.55
SCA × D	5	133.22**	52.14	27.39	3.29	0.74	1.64*
R × D	10	74.81**	94.91**	188.41**	2.93	2.91**	1.46*
Error	120	28.99	30.09	69.16	3.11	0.74	0.71
CV, %		15.94	15.31	11.42	27.12	19.86	9.38

*,** Significant at the 5% and 1% levels of probability, respectively

stated otherwise. The pH of the medium was adjusted to 5.8 before sterilization by autoclaving for 17 min at 121 °C and at a pressure of 15 psi. Immature embryos were cultured on the above media with two concentrations of 2,4-D (0.5 and 1.0 mg/l) for callus induction and somatic embryo initiation. All calli were then transferred to the same medium but with 0.1 mg/l 2,4-D for shoot and root development 4 weeks after somatic embryo initiation. The cultures were placed in controlled incubators under continuous white fluorescent light condition at 21 °C ± 2 °C. Light condition was found to favor somatic embryogenesis induction in our plant material.

Experimental design and data analysis

The experiment was conducted in a completely randomized design with 20 genotypes, two 2,4-D concentrations (0.5 and 1.0 mg/l) and four replications. Ten immature embryos in one petri dish comprised one experimental unit. Embryogenesis, shoot, and root induction frequencies (EIF, SIF, and RIF, respectively) were measured by percentage, based on the number of calli producing somatic embryos, shoots, and roots (EN, SN, and RN, respectively) divided by the total number of embryos cultured in an experimental unit. The weighted average numbers of somatic embryos, shoots, and roots were measured as the total number of somatic embryos, shoots, and roots divided by the number of responsive calli in an experimental unit. Embryogenesis frequency and the weighted average number of somatic embryos were obtained after 4 weeks of callus initiation. Shoot and root induction frequencies as well as the weighted average numbers of shoots and roots were collected 5 weeks after being transferred to the medium containing 0.1 mg/l 2,4-D. The statistical analyses were performed according to Griffing's method 3, model 1 (Griffing 1956). The percentage data for EIF, SIF, and RIF were transformed by using arcsin $\sqrt{y}$ transformation prior to statistical analyses.

Table 3. Proportions (%) of total cross sum of squares due to GCA, SCA, and reciprocal (R) effects for the six characters. Sums of squares for embryogenesis, shoot, and root induction frequencies (EIF, SIF, and RIF, respectively) were estimated from transformed values (arcsin $\sqrt{y}$)

2,4-D	Effect	EIF	SIF	RIF	EN	SN	RN
1.0 mg/l	GCA	84.81	85.53	61.25	34.33	77.53	51.43
	SCA	6.72	6.03	10.07	18.46	11.15	15.50
	R	8.48	8.43	28.69	47.19	13.23	33.23
0.5 mg/l	GCA	80.60	75.60	53.45	45.37	61.17	40.00
	SCA	5.60	4.54	8.99	16.80	6.63	5.46
	R	13.80	19.86	37.56	37.82	32.22	54.49
Combined	GCA	87.95	83.95	63.71	41.39	73.13	51.16
	SCA	3.52	4.74	10.08	16.86	8.56	5.78
	R	8.53	11.32	26.21	41.76	18.32	43.16

Results

The statistical analysis within each 2,4-D level (Table 1) indicated that significant variation among crosses existed for all characters tested. Diallel analyses showed that general combining ability (GCA), specific combining ability (SCA), reciprocal, and maternal effects were significant for most instances. Mean squares from the combined analysis of the diallel crosses at two levels of 2,4-D (Table 2) show that the interactions between 2,4-D concentrations, GCA, SCA, and reciprocal effects were also statistically significant in several cases. This suggests that the magnitude of these genetic effects changed with different 2,4-D levels in the culture medium.

The relative importance of GCA, SCA, and reciprocal effects for the six characters was further analyzed. Table 3 presents the percentage of cross sum of squares accounted for by GCA, SCA, and reciprocal effects. The proportion due to GCA effect was much higher than SCA effect in all cases. The proportion due to GCA effect was also higher than reciprocal effect except for EN in combined and 1.0 mg/l 2,4-D treatments, and RN in 0.5 mg/l 2,4-D concentration. More than 80% of the observed genotypic variation in somatic embryogenesis induction frequency was due to additive effects.

The estimates of GCA and maternal effects of each parental line on the six characters are shown in Tables 4–6. Positive values indicate a contribution towards responses, while negative values represent the opposite. Among the genotypes examined, only 'Mit' had significant positive GCA effects on the six characters in separate analysis by each 2,4-D concentration and combined analysis, showing that it was the best combiner for the in vitro responses studied. The other four parental lines showed negative GCA effects in most cases. Generally, the magnitude and sign of the GCA effect of each line is in agreement with our previous observations on their individual performance *per se*. This indicated that initial selection of parents for hybrid combinations may be largely based on the embryogenesis and organ regeneration responses of the lines. 'Mit', however, also had significant positive maternal effect except for RIF and RN. Other lines showed maternal effects, but directions varied. This indicates that cytoplasmic influences are involved in the inheritance of embryogenesis and organ regeneration.

Discussion

The five parents for the diallel crosses were chosen because of their differences in the characters under study. Thus, the results of this study in a strict statistical sense apply only to these specific lines. However, these lines do represent a reasonable sample of the hard red winter wheat cultivars available for embryogenesis and plant regeneration work; therefore, some extrapolation of the finding may be appropriate.

The GCA effect was significant for the six characters studied in our experiment; thus, the average value of a line was important in predicting the response of a given cross. The additive genetic effects were most important in the genetic control of somatic embryogenesis induction, which is a crucial factor for establishing an efficient culture system. Our results indicate that with this set of inbreds, progress can be made by selecting for highly embryogenic and regenerable winter wheat lines. Significant GCA effect has been reported by Lazar et al. (1984) for the inheritance of regeneration frequency of anther cultures in a diallel cross of five spring wheat cultivars. Similar results have been found in hexaploid triticale for embryogenesis and plant regeneration (Charmet and Bernard 1984), in maize for shoot and root forming capacities (Beckert and Qing 1984), and in tomato for shoot-forming capacity (Frankenberger et al. 1981).

Not all of the variation among crosses was attributable to additivity, since the SCA effect, the hybrid deviation from the averaged GCA effects of two parents, was also significant, except for RIF and RN in 0.5 mg/l 2,4-D level in this study. Thus, non-additive genetic effects (dominance and epistasis) may play a role in the expression of these characters. These results are similar to the previous reports (Beckert and Qing 1984; Charmet and Bernard 1984) that embryogenesis and organ regeneration were primarily under the control of additive gene system but also with considerable importance of SCA effect.

Both GCA and SCA effects were significant in most cases, indicating that additive and non-additive genetic effects are important for the characters examined. However, the proportion of cross sum of squares due to GCA was always much larger than SCA in every case, especial-

Table 4. Estimates of general combining ability (GCA) and maternal (Mat) effects for each parent in 1.0 mg/l 2,4-D. Effects for embryogenesis, shoot, and root induction frequencies (EIF, SIF, and RIF, respectively) were estimated from transformed values (arcsin $\sqrt{y}$)

Parent	EIF		SIF		RIF		EN		SN		RN	
	GCA	Mat	GCA	Mat	GCA	Mat	GCA	Mat	GCA	Mat	GCA	Mat
Arkan	−0.36	−4.99	−2.47**	−3.40	−2.24	9.83	0.02	−2.53*	0.09	−1.23*	−0.48**	−1.06
Mit	17.40**	9.72**	21.15**	12.45**	12.53**	−11.99*	1.88**	4.59**	2.10**	2.35**	1.05**	1.07
Sturdy	−6.68**	3.23	−8.23**	5.94	−8.45**	11.28*	−0.41	−1.55	−0.30	0.63	0.04	0.48
Tam101	−6.01**	3.99	−6.70**	−1.03	−0.79	10.57	−0.84**	−3.23**	−0.69**	0.00	−0.23	1.55*
Vona	−4.34**	−11.95**	−3.75**	−13.95**	−1.04	−19.69**	−0.64*	2.71*	−1.21**	−1.75**	−0.39*	−2.04**
SE	0.71	2.73	0.84	3.24	1.40	5.43	0.29	1.11	0.16	0.61	0.16	0.61

*, ** Significant at the 5% and 1% levels of probability, respectively

Table 5. Estimates of general combining ability (GCA) and maternal (Mat) effects for each parent in 0.5 mg/l 2,4-D. Effects for embryogenesis, shoot, and root induction frequencies (EIF, SIF, and RIF, respectively) were estimated from transformed values (arcsin $\sqrt{y}$)

Parent	EIF		SIF		RIF		EN		SN		RN	
	GCA	Mat	GCA	Mat	GCA	Mat	GCA	Mat	GCA	Mat	GCA	Mat
Arkan	−2.19	−8.46	−4.18**	−12.49**	−5.37**	3.25	−0.95**	−2.33	−0.64**	−1.93**	−0.62**	−2.94**
Mit	17.44**	18.30**	18.99**	26.61**	11.67**	6.91	2.52**	5.19**	2.18**	5.66**	0.99**	2.60**
Sturdy	−4.93**	−10.71*	−4.32**	−7.22	−5.34**	6.67	−0.64	−1.98	0.01	−1.36*	−0.03	0.20
Tam101	−3.50**	−3.23	−4.16**	−9.46*	1.64	0.45	−0.23	−3.70**	−0.47**	−2.39**	0.16	1.06
Vona	−6.82**	4.10	−6.33**	2.57	−2.61	−17.28**	−0.70*	2.81*	−1.07**	0.01	−0.49**	−0.93
SE	1.20	4.64	1.14	4.43	1.63	6.30	0.35	1.37	0.16	0.61	0.15	0.58

*, ** Significant at the 5% and 1% levels of probability, respectively

Table 6. Estimates of general combining ability (GCA) and maternal (Mat) effects for each parent in a combined analysis of two 2,4-D levels. Effects for embryogenesis, shoot, and root induction frequencies (EIF, SIF, and RIF, respectively) were estimated from transformed values (arcsin $\sqrt{y}$)

Parent	EIF		SIF		RIF		EN		SN		RN	
	GCA	Mat	GCA	Mat	GCA	Mat	GCA	Mat	GCA	Mat	GCA	Mat
Arkan	−1.27	−6.72*	−3.32**	−7.95**	−3.81**	6.54	−0.47*	−2.43**	−0.28*	−1.58**	−0.55**	−2.00**
Mit	17.42**	14.01**	20.07**	19.53**	12.10**	−2.54	2.20**	4.89**	2.14**	4.01**	1.02**	1.84**
Sturdy	−5.81**	−3.74	−6.27**	−0.64	−6.89**	8.97*	−0.52*	−1.76*	−0.14	−0.37	0.01	0.34
Tam101	−4.76**	0.38	−5.43**	−5.24	0.42	5.51	−0.54*	−3.46**	−0.58**	−1.19**	−0.03	1.31*
Vona	−5.58**	−3.93	−5.04**	−5.70*	−1.82	−18.48**	−0.67**	2.76**	−1.14**	−0.87*	−0.44**	−1.48**
SE	0.70	2.69	0.71	2.74	1.07	4.16	0.23	0.88	0.11	0.43	0.11	0.42

*, ** Significant at the 5% and 1% levels of probability, respectively

ly for EIF, SIF, and SN. It may be concluded that additive genetic effects are more important than non-additive genetic effects in determining the inheritance of the observed characters. Predominance of GCA effect suggests that mean parental performance should be a useful indicator of mean cross performance for embryogenesis and organ regeneration in winter wheat. Greater emphasis should then be placed on selection within hybrid populations derived from highly responsive parents.

The significance of reciprocal and maternal effects suggests that the variation observed in this experiment was not only due to nuclear genetic effects. Reciprocal differences for in vitro responses are generally attributed to cytoplasmic factors, physiological characteristics of maternal plants, or specific interactions between cytoplasmic and nuclear genetic factors. Maternal effects originate from differences in cytoplasm, usually involving DNA in replicating organelles such as mitochondria, or from differences in the maternal environment provided to the developing embryos by the female parent. Our results agree with other reports which have suggested reciprocal or cytoplasmic effects on embryogenesis and regeneration from plant tissue cultures (Keyes et al. 1980; Beckert and Qing 1984; Charmet and Bernard 1984; Tomes and Smith 1985; Mathias et al. 1986; Powell and Caligari 1987). Due to the presence of maternal effects, it may be critical to use a suitable genotype as a female parent in a selection program using standard breeding methods.

The cross × 2,4-D concentration (C × D) interaction was significant for all characters tested due to differences in magnitude and direction of response under the two 2,4-D concentrations. A partitioning of the C × D interaction resulted in the GCA × D, the SCA × D, and the R × D interactions being significant for several characters. The lack of stability of the hybrid responses in the two 2,4-D concentrations suggests that the in vitro conditions had a significant effect on the various genotypes in this study. For example, 'Arkan' had significant negative GCA values in 0.5 mg/l 2,4-D but had nonsignificant values in 1.0 mg/l 2,4-D for EN and SN. Similar cases were detected in 'Sturdy' and 'Tam 101' for some characters. The smaller magnitude of GCA × D compared to GCA mean squares further suggests that the interaction effects may be of relatively minor importance for the characters.

The results from this experiment agreed with other reports (Maddock et al. 1983; Sears and Deckard 1982; Datta and Wenzel 1987) that 2,4-D concentration influenced somatic embryogenesis and organ regeneration in wheat. Our results indicate that the successful establishment of highly embryogenic and regenerable wheat lines may depend upon the choice of genetic materials as well as in vitro culture conditions, and that genetic improvement is possible through conventional breeding and selection methodologies for embryogenesis and regeneration capabilities in wheat, as demonstrated in alfalfa (Bingham et al. 1975) and maize (Petolino et al. 1988).

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References

Beckert M, Qing CM (1984) Results of a diallel trial and a breeding experiment for in vitro aptitude in maize. Theor Appl Genet 68:247–251

Bingham ET, Hurley LV, Kaatz DM, Saunders JW (1975) Breeding alfalfa which regenerates from callus tissue in culture. Crop Sci 15:719–721

Bullock WP, Baenziger PS (1982) Anther culture of wheat (*Triticum aestivum* L.) F_1's and their reciprocal crosses. Theor Appl Genet 62:155–159

Caligari PDS, Powell W, Goodall V (1987) The in vitro genetics of barley (Hordeum vulgare L.): genetic analysis of immature embryo response to 2,4-dichlorophenoxyacetic acid. Heredity 59:285–292

Charmet G, Bernard S (1984) Diallel analysis of androgenetic plant production in hexaploid Triticale (*X. triticosecale*, Wittmack). Theor Appl Genet 69:55–61

Datta SK, Wenzel G (1987) Isolated microspore derived plant formation via embryogenesis in *Triticum aestivum* L. Plant Sci 48:49–54

Frankenberger EA, Hasegawa PM, Tigchelaar EC (1981) Diallel analysis of shoot-forming capacity among selected tomato genotypes. Z Pflanzenphysiol 102:233–242

Griffing B (1956) Concept of general ans specific combining ability in relation to diallel crossing systems. Aust J Biol Sci 9:463–493

Hanzel JJ, Miller JP, Brickman MA, Fendos E (1985) Genotype and media effects on callus formation and regeneration in barley. Crop Sci 25:27–31

Hodges TK, Kamo KK, Imbrie CW, Becwar MR (1986) Genotype specificity of somatic embryogenesis and regenation in maize. Biotechnology 4:219–223

Keyes GJ, Collins GB, Taylor NL (1980) Genetic variation in tissue culture of red clover. Theor Appl Genet 58:265–271

Lazar MD, Baenziger PS, Schaeffer GW (1984) Combining abilities and heritability of callus formation and plantlet regeneration in wheat (*Triticum aestivum* L.) anther cultures. Theor Appl Genet 68:131–134

Maddock SE, Lancaster VA, Risiott R, Franklin J (1983) Plant regeneration from cultured immature embryos and inflorescences of 25 cultivars of wheat (*Triticum aestivum*). J Exp Bot 34:915–926

Magousson I, Bornman CH (1985) Anatomical observations on somatic embryogenesis from scutellar tissues of immature zygotic embryos of *Triticum aestivum*. Physiol Plant 63: 137–145

Mathias RJ, Fukui K (1986) The effect of specific chromosome and cytoplasm subtitutions on the tissue culture response of wheat (*Triticum aestivum*) callus. Theor Appl Genet 71: 797–800

Mathias RJ, Simpson ES (1986) The interaction of genotype and culture medium on the tissue culture responses of wheat

(*Triticum aestivum* L. em. thell) callus. Plant Cell Tissue Organ Cult 7:31–37

Mathias RJ, Fukui K, Law CN (1986) Cytoplasmic effects on the tissue culture response of wheat (*Triticum aestivum*) callus. Theor Appl Genet 72:70–75

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

Petolino JF, Jones AM, Thompson SA (1988) Selection for increased anther culture response in maize. Theor Appl Genet 76:157–159

Powell W, Caligari PDS (1987) The in vitro genetics of barley (Hordeum vulgare L.): detection and analysis of reciprocal differences for culture response to 2,4-dichlorophenoxyacetic acid. Heredity 59:293–299

Sears RG, Deckard EL (1982) Tissue culture variability in wheat: Callus induction and plant regeneration. Crop Sci 22:546–550

Tomes DT, Smith OS (1985) The effect of parental genotype on initiation of embryogenic callus from elite maize (*Zea mays* L) germplasm. Theor Appl Genet 70:505–509