EFFECT OF THE GENOTYPE AND GAMMA IRRADIATION ON THE ANTHER CULTURES OF A 10 × 10 DIALLEL CROSS OF WHEAT

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Abstract

Anther culture responsiveness, irradiation effect and reciprocal effect were evaluated on ten genotypes (V1-V10) and a 101 × 0 diallel cross. Gamma irradiation dose of 100 Gy was applied to seeds of parents and F_1 cross from which the donor plants were grown. Non-irradiated donor plants were also used for comparison. Anthers were plated on potato-2 callus induction medium and calli formed were transferred to MS medium supplemented with sucrose (3%), indolacetic acid (1.0 mg/L), kinetin (1.0 mg/L), inositol (100 mg/L) and solidified with agar (0.7%). Genotypes showed big differences for callus induction, plant regeneration and anther culturability rate. The most responsive materials were V2, V10 and V5 with 76.0, 27.4 and 10.8 green plants per 100 anthers respectively. No irradiation effect was found for the parents nor the F_1 crosses on the pooled data. Mean anther culture response of specific genotypes showed that irradiation significantly increased anther culturability rate of V3 from 0.1 to 27.6 green plants per 100 anthers. No reciprocal effect was observed.

1. INTRODUCTION

Wheat has been an important crop in feeding the population of Guatemala. Its improvement began in 1958 with close collaboration of CIMMYT and 26 varieties have been released since then. Recently, the anther culture and mutation induction techniques, with a great potential for improving varieties, were included in the wheat breeding program.

In the anther culture approach, the immature pollen grains form embryos or calli which become plants when transferred to regeneration medium. Most of the regenerated plants are sterile haploids but in some species like *Triticum* a spontaneous chromosome doubling occurs during callus development and plant regeneration.

The main advantage for the breeding programs is the time saved by obtaining homozygous plants from the F_1 hybrids. Besides, the selection efficiency increases due to visible expression of recessive alleles. Thus, smaller populations are needed as compared with the pedigree method.

The main problem in using this method is the low culturability of the anthers. One way to solve this is by selecting genotypes with high androgenetic response. However, it is necessary to develop or improve the procedures that produce haploidy in the important crops.

The first production of wheat haploid plants using anther culture was simultaneously reported in China and France. Since then, many studies have proven that the pathway from microspores to fertile plants is influenced by several factors as the genotype [1], the developmental stage of the microspore [2], as well as the physiological conditions of the donor plants and the culture medium.

The genotype is the most important factor affecting anther culture. Lazar *et al.* [1], in wheat, have estimated the genetic parameters, for the two principal characters, callus and

plantlet induction rates in a diallel analysis of five parental lines. They found that the total variation effect is mainly due to genetic factors with a preponderance of additive effects, but also with significant dominance (specific combining ability) and reciprocal effects. The results of the past years strongly suggest that the organization of the androgenetic process is under the control of alleles with quantitative effects, it is heritable and its level of expression can be enhanced by selection.

It is thus clear that success in the production of doubled haploid lines from the F_1 progenies will depend largely on the selection of the best responding genotypes with high specific combining ability.

Mutation induction has become an established tool in plant breeding to supplement existing germplasm variation and for improving cultivars in certain specific traits. Limitations arise mainly from the large mutagenized populations to be screened and from the unsatisfactory selection methods. Both limitations may be eased to some extent by advances in techniques of plant in-vitro culture [3].

When induced mutations and haploid production are combined, recessive alleles induced by a mutagen before or during the haploid stage will be homozygous in the doubled, diploid phase, and therefore phenotypically expressed [4].

Low-dose gamma irradiation of fresh explants can significantly improve regeneration from anther cultures in wheat and may stimulate a low frequency of regeneration in an otherwise non-responsive cultivar [5].

The aim of this study is to characterize the responsiveness of ten elite wheat genotypes to anther culture, the effect of gamma irradiation on their response and the reciprocal effect on 86 F_1 hybrid combinations from a 10 × 10 diallel cross. This should be of great help in selecting genotypes for breeding using the doubled haploid (DH) procedures.

2. MATERIALS AND METHODS

The Guatemalan variety ICTA Cumpale (V1), eight advanced lines from the crosses CMBW (V2), CM76694 (V3), CM90722 (V4), CM92909 (V5), CM76635 (V6, V7), CM113143 (V8), CM81812 (V9) introduced from CIMMYT, and an advanced line from the cross CG12334 (V10) were used by the National Wheat Program for a diallel cross. Ten seeds from each entry were taken for the study. Half of them were irradiated at a dose of 100 Gy with a ^{60}Co source at a dose rate of 7.17 Gy min^{-1}. All the plants were grown in 20 cm pots under natural light; the temperature varied between 18 and 28°C. Spikes were collected when the microspores were in the uninucleate stage and were subjected to a cold stress for 4 days at 4°C. Anthers were plated on potato 2 medium and kept in the dark at 25-27°C. After 4-6 weeks, calli were transferred to an MS regeneration medium with 1 mg/L indolacetic acid, 1 mg/L kinetin, 30 g/L sucrose and solidified with 7 g/L agar under 16 hrs photoperiod at 25-27°C.

Callus induction rate was considered as the number of calli formed per one hundred plated anthers. Plant regeneration rate was considered as the number of green plants regenerated per one hundred calli transferred and anther culturability as the number of green plants regenerated per one hundred plated anthers. The means for the ten genotypes as well as the 86 F_1 hybrids from the 10 × 10 diallel cross were compared.

3. RESULTS

The genotypes demonstrated a wide range of responses to anther culture and no significant effect of the irradiation treatment (Table I). In the non-irradiated controls, the callus induction rate varied from 1.0 to 146.7 with a mean value of 15.5 calli per 100 anthers. In the irradiated group, the callus induction rate varied from 0.6 to 60.0 with a mean value of 17.1 calli per 100 anthers. The plant regeneration rate varied from 1.6 to 128.6 with a mean value of 0.5 green plants regenerated per 100 calli transferred in the non-irradiated treatment and from 0.0 to 98.6 with a mean value of 0.4 green plants regenerated per 100 calli transferred in the irradiated treatment. Anther culturability rate also showed a wide range of responses among genotypes and no significant difference of the irradiation treatment. For the controls, the rate varied from 0.1 to 76.0 with a mean value of 0.1 green plants regenerated per 100 anthers and from 0.0 to 27.6 with a mean value of 0.1 green plants regenerated per 100 anthers for the irradiated group.

TABLE I. A COMPARISON OF ANTHER CULTURE RESPONSE OF THE IRRADIATED WHEAT GENOTYPES AND THEIR CONTROLS

Genotype	Plated anthers No.	Calli formed No.	Callus induction (%)	Calli transferred No.	Green plants No.	Plant regeneration (%)	Anther culturability (%)
Control							
V1	250	50	20.0	49	16	32.7	6.4
V2	150	220	146.7	220	114	51.8	76.0
V3	800	72	9.0	64	1	1.6	0.1
V4	700	7	1.0	7	9	128.6	1.3
V5	650	192	29.5	160	35	21.9	5.4
V6	250	44	17.6	40	27	67.5	10.8
V7	1250	90	7.2	79	69	87.3	5.5
V8	1050	44	4.2	6	6	100.0	0.6
V9	350	48	13.7	41	10	24.4	2.9
V10	350	120	34.3	120	96	80.0	27.4
Irradiated							
V1	300	55	18.3	53	39	73.6	13.0
V2	250	150	60.0	140	31	22.1	12.4
V3	250	80	32.0	70	69	98.6	27.6
V4	350	2	0.6	2	0	0.0	0.0
V5	400	55	13.8	45	11	24.4	2.8
V6	350	33	9.4	33	0	0.0	0.0
V7	350	41	11.7	41	21	51.2	6.0
V8	450	17	3.8	17	0	0.0	0.0
V9	200	4	2.0	2	0	0.0	0.0
V10	400	126	31.5	99	46	46.5	11.5

Based on anther culturability, 3 genotypes were classified as highly responsive, namely: V2, V10 and V6; 3 were classified as intermediate: V1, V7 and V5, while 4 were classified as poorly responsive, V9, V4, V8 and V3.

The same trend was observed for the F_1 hybrids but in this case, the range of the anther culture response was narrower than that of the ten parents (Table II). The callus induction rate when pooled by the same parent varied from 6.1 to 31.7 with a mean value of 18.3 calli per 100 anthers in the non-irradiated treatment. For the irradiated treatment, the range was

between 9.8 and 28.4 with a mean value of 18.0 calli per 100 anthers. Plant regeneration rate varied from 11.9 to 81.5 with a mean value of 0.5 green plants per 100 calli transferred for the non-irradiated treatment and from 22.1 to 62.4 with a mean value of 0.4 green plants per 100 calli transferred for the irradiated group. Anther culturability for the controls, when pooled by same parent, ranged from 1.8 to 18.4 with a mean value of 0.1 green plants per 100 anthers and from 2.2 to 11.6 with a mean value of 0.1 green plants per 100 anthers for the irradiated treatment.

Based on anther culturability, hybrids involving V2, V5 and V10 were classified as highly responsive, hybrids with V1, V3, V6, V7 and V9 were classified as intermediate and hybrids involving V8 and V4 were classified as poorly responsive.

TABLE II. MEAN CALLUS INDUCTION, PLANT REGENERATION AND ANTHER CULTURABILITY OF 86 F_1 HYBRIDS POOLED BY THE SAME PARENT

Parent	Plated anthers No.	Callus induction (%)	Plant regeneration (%)	Anther culturability (%)
Control				
V1	2850	15.4	44.8	6.4
V2	2500	27.0	70.8	18.0
V3	2050	15.6	50.2	6.0
V4	2600	6.1	81.5	4.2
V5	1750	31.7	53.4	14.8
V6	1300	10.8	77.5	7.2
V7	2100	22.2	44.1	8.4
V8	1800	16.1	11.9	1.8
V9	2050	12.3	50.2	5.9
V10	2100	26.7	55.9	14.3
Irradiated				
V1	2950	15.0	42.8	5.8
V2	2700	24.4	45.5	9.8
V3	2100	18.5	62.4	10.3
V4	2650	12.0	44.1	4.4
V5	1600	28.4	32.4	8.3
V6	1250	9.8	22.1	2.2
V7	1900	18.5	41.7	7.1
V8	1550	19.6	27.9	4.7
V9	2150	12.0	41.0	4.0
V10	2200	21.9	60.7	11.6

Anther culture responsiveness of the F_1 pooled as male and female parents did not show significant differences among genotypes (Table III). When pooled by female parents, the mean callus induction rate of the F_1's ranged from 9.1 to 30.1 calli per 100 anthers and from 7.2 to 29.5 when used as male parents. Plant regeneration rate varied from 19.7 to 58.7 green plants per 100 calli, when pooled by female parents and from 18.9 to 70.8 when pooled by the males. Anther culturability ranged from 3.2 to 13.7 green plants per 100 anthers when pooled by female parents and from 2.5 to 16.5 when pooled by the male parents.

Based on anther culturability rate, three genotypes were classified as highly responsive when used both as female or male parent: V2, V10 and V5.

TABLE III. MEAN CALLUS INDUCTION, PLANT REGENERATION AND ANTHER CULTURABILITY OF 86 F_1 HYBRIDS POOLED BY THE SAME FEMALE OR MALE PARENTS

Parent	Plated anthers No.	Callus induction (%)	Plant regeneration (%)	Anther culturability (%)
Used as female				
V1	5800	15.2	43.8	6.1
V2	5200	25.8	58.7	13.7
V3	4150	17.1	57.3	8.2
V4	5250	9.1	56.8	4.3
V5	3350	30.1	43.8	11.7
V6	2550	10.3	49.6	4.7
V7	4000	20.4	43.0	7.8
V8	3350	17.7	19.7	3.2
V9	4200	12.1	45.9	4.9
V10	4300	24.3	58.0	12.9
Used as male				
V1	4800	18.1	70.8	11.8
V2	3750	20.6	49.6	8.7
V3	5600	24.0	43.1	9.2
V4	5600	8.7	39.7	3.1
V5	4100	24.8	36.4	8.3
V6	3400	15.6	54.9	8.0
V7	3700	15.1	41.8	5.4
V8	3600	15.9	18.9	2.5
V9	3400	7.2	67.3	4.4
V10	4200	29.5	61.5	16.5

4. DISCUSSION

The genetic effect on anther culturability of wheat [1] was confirmed in this report. Big differences were found among the genotypes used. Three out of ten were classified as highly responsive, four as intermediate and three as poor. These results also confirm that wheat anther culture breeding depends largely on the selection of the best responding genotypes.

The overall analysis did not show any difference between non-irradiated and irradiated parents in the three traits studied (callus induction, plant regeneration and anther culturability rate). However, a significant difference was observed for the genotype V3, whose anther culturability rate rose from 0.1 to 27.6 green plants per 100 anthers when irradiated. Plant regeneration rate for this genotype was also raised from 1.6 to 98.6 and its callus induction, from 9.0 to 32.0 calli per 100 anthers, by irradiation.

These results may be due to the fact that the irradiation effect is dose-dependent and its extent varies with genotype [5]. In the same way, the overall analysis of the F_1 hybrids did not show any difference between them.

Reciprocal differences appeared not significant, suggesting no cytoplasmic effect on the anther culture response. Such reciprocal cross-specific responses of the F_1 progeny were previously shown by Bullock *et al.* [6]. Some slight differences observed on the mean anther culture response by pooling genotypes as male and female may be due to G × E interactions.

The information obtained in this study has practical applications in employing anther culture in wheat variety improvement in order to obtain large number of regenerated DH lines for evaluation and selection.

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