

Genetic Contribution of Parental Genotypes on Anther Culture Response of Bread Wheat F₁ Hybrids

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ABSTRACT

Anther culture response of four wheat genotypes and their 12 F₁ crosses using a 4 × 4 complete diallel set of crosses was investigated. Results indicated that considerable genetic variation among tested genotypes was observed. The best performance of the two crosses; Lin-A × Misr-1 and Misr-2 × Gemmeiza-11 had the parents; Line-A and Gemmeiza-11, which exhibited a good performance for responding anthers of F₁ and reciprocals. The highest frequencies of green plantlets were achieved from the cross Misr-2 × Gemmeiza-11 and its reciprocal, while the cross Misr-2 × Misr-1 did not regenerate green plantlets. Generally, crosses showed a better response in anther culture than their parental genotypes. Significant and positive heterotic effects were observed in some crosses for all studied traits. General and specific combining ability effects were highly significant and desirable for anther culture response. The good general combiners for green plant regeneration were Line-A and Gemmeiza-11 as they attained highly significant and positive general combining ability effects. The best cross-combinations for callus induction and plant regeneration were Line-A × Misr-1 and Misr-2 × Gemmeiza-11. Reciprocal effects were highly significant for all studied traits. The SCA variance had the higher percentage of genetic contribution for all studied traits than GCA and reciprocal variances. The results also indicate that the parents, which give rise to highly responsive hybrids, can be identified and that genetic improvement of wheat is possible through selection.

Key words: *Triticum aestivum* L., anther culture, general and specific combining ability.

Introduction

Anther culture of wheat (*Triticum aestivum* L.) is a useful tool for plant breeders, which offers a rapid method of creating homozygous lines from heterozygous genotypes within a short time and allows for an increase in selection efficiency due to better discrimination between genotypes within any generation and efficient retention of desirable alleles in later generations (Ghaemi *et al.*, 1995 and Adamski *et al.*, 2014).

Anther culture is accomplished and green plants are obtained in two steps: the first step involves the induction of embryogenic calli from microspores and the next step deals with the regeneration of green plants from the calli (Bishnoi *et al.*, 2000). However, the low percentage of callus induction and green plant regeneration of most wheat cultivars has limited the application of anther culture techniques in breeding programmes. Especially, some breeding materials showing good agronomic performance are recalcitrant for production of green plants. Anther culture has not yet become a routine technique for all wheat cultivars and their hybrids, although it is a good level in some genotypes (Orlov *et al.*, 1993). For this reason, more information on the combining ability of breeding material for anther culture response would be extremely useful for the improvement of anther culture effectiveness (Dagustu, 2008).

Haploid plants are of great importance, which are used in achieving homozygosity in quick way, and facilitating genetic and breeding researches (El-Hennawy *et al.*, 2011). The success of anther culture ability in wheat, as other crops, is found to be influenced by genotype (Andersen *et al.*, 1987), donor plant growth conditions (Orshinsky and Sadasivaiah, 1997), the developmental stage of microspores (Haggag and El-Hennawy, 1996), pre-culture treatments, media components (Lazaridou *et al.*, 2005) and the interactions among these factors (Lazar *et al.*, 1984).

The genes governing traits can be transmitted from parents to progenies via the nucleus (chromosomes) or the cytoplasm (mitochondrial DNA or chloroplast DNA). Cytoplasmic organelles such as mitochondria and chloroplasts, which contain their own DNA and genes, are almost entirely inherited through the female parent and hence give rise to permanent differences between them over many generations of selfing or outcrossing. Maternal effects are important because they can bias the means and variances of families and mislead breeders in their attempts to understand the genetics of a given quantitative trait (Kearsey and Pooni, 1996).

Information on the reciprocal or maternal effects on anther culture response of wheat is limited, although much useful genetic information on nuclear genetic control has been reported (Yildirim *et al.*, 2008). All androgenic component traits of anther culture have been found to be polygenically controlled and are independently inherited traits (Ekiz and Konzak, 1994). Most of the studies on the inheritance of anther culture response in wheat have focused only on nuclear genetic control and have not examined the reciprocal effects

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(Moinei *et al.*, 1997). Chaudhary *et al.* (2003) reported that maternal and cytoplasmic effects are not important for anther culture response. However, Ghaemi *et al.* (1995) reported significant reciprocal effects affecting the anther culture responses of wheat.

The use of one parent as male or female could lead to change in the production of green plants from the F₁ hybrids and screening of inbred lines for response to anther culture, without reciprocal effects, could decrease the utilization of breeding material (Yildirim *et al.*, 2008). Furthermore, Khiabani *et al.*, (2008) found heterotic effect for plant regeneration. Zamani *et al.* (2003) stated that the *in vitro* anther culture response of a hybrid was affected by both parents. The positive effect of cytoplasm in anther culture response has been demonstrated in wheat (Ghaemi *et al.*, 1995). This study was conducted to estimate the effect of parental genotypes and genetic contribution (nuclear and cytoplasmic factors) on the androgenic capacity of F₁ hybrids.

Materials and Methods

The present investigation was carried out at the Cell and Tissue Culture Laboratory as well as the Experimental Farm of the Agronomy Department, Fac. of Agric., Al-Azhar Univ., Nasr City, Cairo. Four genotypes of bread wheat representing a wide range of diversity for several traits were used for this study. Three local varieties *vis.*, Misr-1, Misr-2 and Gemmeiza-11 as well as the Line-A were used in this study. The Line-A was obtained from Prof. Dr. M. A. El-Hennawy, Agronomy Dept., Fac. of Agric., Al-Azhar Univ. In 2011/12 growing season, the four parents were crossed in a diallel mating design, including reciprocals. The parents and their 12 F₁ hybrids were grown in the Experimental Farm in 2012/13 season to obtain the needed anthers.

Whole tillers at boot stage were collected when most microspores were at the mid- to late uninucleate stage of development, as assessed by acetocarmine staining of selected squashed anthers. The tips of the spikes were at the ligule of the penultimate leaf was used as an indicator of this stage. Tillers with spikes at this stage were clipped off at ground level and tagged. Then, they were put in water and maintained for 6-8 days at 4 °C in the dark. After cold pretreatment, the spikes inside flag leaves were surface sterilized with 20 % chlorax solution for 7 min. and rinsed 3-4 times in sterile water. Anthers were aseptically dissected out and cultures in jars containing the N6 induction medium of anther culture (Chu, 1978) supplemented with 2 mg/L 2,4-D, 1 mg/L kinetin, 90 gm /L sucrose and 7 gm/L agar. Thirty anthers from each spike were plated in each jar. An average of 20 jar (replications) for each genotype was cultured. These jars were incubated first for 5-6 weeks in darkness at 28 °C. Completely randomized design was applied in this experiment with 16 genotypes.

Embryoids / callus induced from the anthers were transferred to jars containing MS regeneration medium (Murashige and Skoog, 1962) supplemented with 0.5 mg/L NAA, 0.5 mg/L kinetin, 30 gm/L sucrose and 6 g/L agar. These jars placed under cool white fluorescent lamps for 16 h and temperature 25 -27 °C. The number of green and albino plantlets regenerated in each jar was counted after about 30 d depending on plant development.

Green plantlets with adequate root formation were transplanted to small pots with mixture of soil, sand and compost, under plastic cover for three weeks in a growth chamber maintained at 18 °C and 16 h light per day. The data recorded on all the traits (callus induction, embryogenic callus induction, number of green plantlets and number of albino plantlets) were subjected to analysis of variance (Steel and Torrie, 1980) to determine the significant differences among genotypes. Heterotic effects were computed relative to better parent for all studied traits. The diallel analysis was performed conducted according to Griffing's (1956) method 1 (including parents and reciprocals) and model 1.

Results and discussion

Effect of parental genotypes on anther culture response of F₁ hybrids:

The successful application of anther culture in wheat breeding programs depends on the good androgenic response of genotypes and the high frequency of chromosome doubling. The 12 F₁ crosses and their parents were evaluated for response to anther culturing and capability of plant regeneration. Significant differences were recorded among the four wheat genotypes and their crosses. Previous studies on anther culture have also shown considerable genetic differences between genotypes for some of the traits of anther culture (Orlov *et al.*, 1993). Genetic factors for all anther culture studied traits were highly significant (Table 1), revealing the presence of genetic diversity in the material used for all studied traits.

The mean values of all studied traits are summarized in Table (2). The two parents; Line-A and Gemmeiza-11 exceeded other genotypes in terms of responding anthers and embryogenic callus. These two genotypes also had a good level of response in number of green plantlets produced per 100 anthers, which was significantly higher than other two genotypes (Table 2). Thus, the Line-A and Gemmeiza-11 exhibited the best performance (7.83 and 8.67 green plantlets per 100 anthers, respectively) in anther culture. The cultivar Misr-1 exhibited the lowest response (2.00 green plantlets per 100 anthers), while it did not regenerate albino plantlets from tested callus cultures.

Table 1: Analysis of variance for anther culture traits of parents and their 4 × 4 full diallel crosses of bread wheat.

S.O.V.	d.f.	Callus induction (%)	Embryogenic callus induction (%)	No. of regenerated plants /100 anthers	
				Green	Albino
Genotypes	15	34.25**	8.936**	31.78**	10.42**
Parent (P)	3	30.82**	15.950**	18.28**	16.15**
Crosses (C)	11	33.76**	7.014**	37.35**	8.95**
P. vs. C	1	49.96**	9.042**	11.05**	9.40**
GCA	3	1.21**	0.576**	2.95**	1.01**
SCA	6	2.49**	0.534**	2.02**	0.549**
Reciprocal	6	1.18**	0.287**	0.48**	0.247**
Error	304	1.75	0.290	1.28	0.90
GCA/SCA		0.06	0.135	0.18	0.23

*and ** significant and high significant at 0.05 and 0.01 probability levels, respectively.

Out of 12 F₁ hybrids studied, two crosses; Lin-A × Misr-1 and Misr-2 × Gemmeiza-11 with the best performance included the parents; Line-A and Gemmeiza-11 with good performance for responding anthers and embryogenic callus of F₁ and reciprocals. The reciprocal cross Misr-1 × Misr-2 gave the highest mean values for the same traits compared with its direct cross. Similar results were found by Zamani *et al.* (2003) and Yildirim *et al.* (2008).

Estimates of better parent heterosis for callus induction and embryogenic callus are presented in Table (3). Four out of twelve crosses showed highly significant and positive heterotic effects for these traits. The highest heterosis value was observed in the cross Misr-2 × Gemmeiza-11. Significant and negative better parent heterosis was detected in seven cases. Ekiz and Konzak (1994) and El-Hennawy *et al.* (2011) also observed that the heterosis for responding anther was present only when the anther donor plants were heterozygous, but disappeared when they were homozygous.

Ability of callus to produce green plantlets varied in relation to the different genotypes. The highest frequencies of green plantlets were achieved from the three crosses; Gemmeiza-11 × Misr-2, Misr-2 × Gemmeiza-11 and Gemmeiza-11 × Misr-1, while the cross Misr-2 × Misr-1 did not regenerate green plantlets from tested callus cultures (Table 2). Two hybrids appeared to have significantly higher green plant yields than their respective better parents. These results indicated that frequency of anthers capable of producing green plantlets was affected by genotypes. Similar results were obtained by Haggag and El-Hennawy (1996), who found genotypic effect on green plant regeneration. It is known that the response to anther culture is strongly genotype dependent (El-Hennawy, 1996 and Barakat *et al.*, 2012). As pointed out in different investigations, androgenic response in wheat was a heritable trait and can be transferred into agriculturally desirable material by crossing (Foroughi-Wehr *et al.*, 1982 and Al-Ashkar, 2013).

Percentage of better parent heterosis for green plant regeneration is presented in Table (3). Relative to their respective better parent values, the cross (Misr-2 × Gemmeiza-11) and its reciprocal cross showed positive and significant heterosis (42.21%). Negative and significant better parent heterosis was observed in six crosses. El-Hennawy *et al.* (2011) reported heterotic effect for green plant regeneration. Bread wheat F₁ hybrids produced higher green microspore derived plants than the best parent, indicating high positive heterosis (Zamani *et al.*, 2003).

Table 2: Anther culture response of 12 F₁ hybrids (F₁'s and reciprocals) and their parents in bread wheat.

Genotypes	Callus induction (%)	Embryogenic callus induction (%)	No. of regenerated plants /100 anthers	
			Green	Albino
Line-A	10.67	7.63	7.83	5.83
Misr-1	1.17	0.56	2.00	0.00
Misr-2	3.83	2.64	3.83	6.67
Gem-11*	7.33	4.11	8.67	5.00
F ₁ 's				
Line-A × Misr-1	16.67	7.25	7.50	10.17
Line-A × Misr-2	5.83	2.88	7.17	8.50
Line-A × Gem-11	7.33	5.00	3.67	5.33
Misr-1 × Misr-2	3.83	1.67	1.50	2.83
Misr-1 × Gem-11	6.17	4.11	4.83	5.17
Misr-2 × Gem-11	18.00	9.44	12.33	5.00
Reciprocals				
Misr-1 × Line-A	8.50	5.67	5.17	7.00
Misr-2 × Line-A	5.00	4.03	8.33	5.00
Gem-11 × Line-A	8.83	5.33	7.67	6.67
Misr-2 × Misr-1	7.67	5.28	0.00	3.50
Gem-11 × Misr-1	8.00	4.78	9.33	2.67
Gem-11 × Misr-2	9.67	4.89	16.67	6.50
L. S. D. 0.05	0.82	0.49	0.70	0.58
L. S. D. 0.01	1.07	0.59	0.92	0.77

* Gemmeiza-11

Regarding albino plants, which is usually uncounted in most similar studies, composes a major problem for the application of anther culture technique in wheat breeding programs. The crosses ranged from 2.67 % for the cross Gemmeiza-11 × Misr-1 to 10.17 % for the cross Line-A × Misr-1 (Table 2). Similar results were found by El-Hennawy *et al.* (2011), who observed genotypic effect on albino plant regeneration. Andersen *et al.* (1987) stated that the formation of albino plants was genetically and environmentally controlled. In addition to the effect of genotype and the duration of maintaining calli in culture, the development stage of microspore at inoculation time as well as the chloroplast DNA deletions may affect occurrence of albinos (Liang *et al.*, 1990).

Estimates of better parent heterosis for albino plants are presented in Table (3). Three out of twelve crosses showed highly significant and positive heterosis values for this trait. When compared with the parents, F₁ and reciprocals were in the range of best parental values for anther culture response. However, some crosses had significantly higher values from better parent values for all traits. The cytoplasm of Misr-1 had positive effects in most of crosses for callus induction and embryogenic callus. Line-A had negative cytoplasmic effects for all anther culture traits except albino plants. Gemmeiza-11 and Misr-2 showed negative and positive cytoplasmic effects for all studied traits.

Table 3: Heterosis as percentage of better parent for the studied traits.

Crosses	Callus induction (%)	Embryogenic callus induction (%)	No. of regenerated plants /100 anthers Green Albino	
F ₁ 's				
Line-A × Misr-1	56.23**	-4.98**	-42.15	00
Line-A × Misr-2	-45.4**	-62.25**	-84.29	45.8**
Line-A × Gem-11	-31.3**	-34.47**	-57.7**	6.6
Misr-1 × Misr-2	0.00	-36.74**	-60.8**	0
Misr-1 × Gem-11	-15.82*	0.00	-44.3**	0
Misr-2 × Gem-11	145.56*	129.68**	42.21**	0
Reciprocals				
Misr-1 × Line-A	-20.33*	-34.56**	-34.0**	0
Misr-2 × Line-A	-53.14**	-47.18**	63.86	0
Gem-11 × Line-A	-17.24**	-30.14**	-11.5**	33.4**
Misr-2 × Misr-1	100.26**	100.00**	-100**	0
Gem-11 × Misr-1	17.49	16.30**	76.12	0
Gem-11 × Misr-2	145.56**	18.97**	42.21**	30.0**

*and ** significant and high significant at 0.05 and 0.01 probability levels, respectively.

Some parents increased or decreased the traits of anther culture response depending on its using as male or female parent in crosses. For example, Line-A and Gemmeiza-11 have the highest values for all investigated traits, but their cytoplasm reduced the performance of all traits. In addition to maternal effects, nucleus × cytoplasm interaction differed for different nucleus and cytoplasm combinations. The cytoplasm of Misr-1 and Misr-2 showed different interactions with different parents. Ekiz and Konzak (1994) and Yildirim *et al.* (2008) observed significant nucleus × cytoplasm interaction effects on anther culture ability of wheat. Differences between reciprocal crosses would indicate that cytoplasmic factors or the maternal tissue could also be involved. Cytoplasmic effects on callus induction and embryogenic callus were more than cytoplasmic effects on green plant number.

The results of the present study are in accordance with previous reports of Barakat *et al.* (2012), who indicated that the genotype played an important role in anther culture. Furthermore, the cross Misr-2 × Gemmeiza-11 and its reciprocal with the highest response in anther culture had parent that exhibited good response. These results indicate that high responding parent could be used to generate responding F₁ hybrids, although there is no guarantee of a high response in the hybrids because the inheritance of anther culture response may be more complicated (Ekhveh *et al.*, 2013). Zamani *et al.* (2003) also reported that a well-responding parent could lead to the production of sufficient green plants for breeding purposes. In addition, the data obtained from this study indicate that hybrids included from one parent with good or intermediate performance in anther culture would be of value for developing an *in-vitro* system with a high production of green plants. Al-Ashkar (2013) also reached the same conclusion.

Genetic contribution on anther culture response of F₁ hybrids:

The general (GCA) and specific (SCA) combining ability effects were highly significant (Table 1), indicating that both additive and non-additive types of gene action were operative for all studied traits. However, the ratio of GCA/SCA was less than unity for all traits, indicating the predominant role of non-additive gene effects in the expression of all traits. Similar results were reported by Lazar *et al.* (1984) and He *et al.* (2006). For all traits, the reciprocal effects were highly significant (Yildirim *et al.*, 2008)

Estimates of GCA effects of the individual parental genotypes for each studied trait are presented in Table (4). The results revealed that parental genotypes exhibited highly significant values for most studied in all cases with either positive or negative significant. The parental Line-A showed highly significant positive effects for all traits, while the parental cv. Misr-1 showed highly significant negative effects for all studied traits. Misr-2 gave highly significant negative effects for most traits. On the other hand, the cv. Gemmeiza-11 seemed to be good combiner for all traits. It is worth to note that Line-A and Gemmeiza-11 were general combiners for green plant regeneration.

Specific combining ability effects (SCA) for F_1 and reciprocal hybrids with respect to the studied traits are given in Table (4). Results indicated that the two crosses; Line-A \times Misr-1 and Misr-2 \times Gemmeiza-11 as well as reciprocal crosses showed positive and highly significant SCA effects for all the studied traits except for No. of regenerated plantlets (green and albino) of reciprocal cross Misr-2 \times Gemmeiza-11, which showed negative and highly significant SCA effects, including good \times poor general combiners parents for all the studied traits. Regarding the cross Line-A \times Misr-2 and its reciprocal, negative and highly significant SCA effects were observed in most cases, while callus induction and albino plant regeneration exhibited positive and significant SCA effects in reciprocal cross only. The cross Line-A \times Misr-1 and its reciprocal showed negative and highly significant SCA effects including good \times good general combiner parents for all the studied traits. Concerning the two crosses; Misr-1 \times Misr-2 and Misr-1 \times Gemmeiza-11 as well as their reciprocals, highly significant SCA values for most studied traits in all cases with either positive or negative significant including poor \times poor and good \times poor general combiners for all studied traits.

The results contrast with those of Tuveesson *et al.*, (1989), Chaudhary *et al.* (2003) and Yildirim *et al.* (2008), who reported that anther culture response of wheat was under the control of nuclear genes without maternal or cytoplasmic effects. Studies by Ghaemi *et al.* (1995) and Dagustu (2008) support our observations of reciprocal differences for all components of anther culture response. Differences between reciprocal crosses would indicate that either cytoplasmic factors (Griffiths *et al.*, 2000) or the maternal tissue (Henry *et al.*, 1994) were effective on anther culture response. Anther culture response appears to be regulated by nuclear genes, cytoplasmic genes or nuclear \times cytoplasmic gene interactions as explained before (Chaudhary *et al.*, 2003 and Dagustu, 2008). However, differences between reciprocal crosses may occur from sampling error, small numbers of regenerants or heterozygosity of mother plants, rather than genetic effects as explained before (Henry *et al.*, 1994).

Table 4: Estimates of general combining ability (GCA), specific combining ability (SCA) and reciprocal effects for the studied traits.

Genotypes	Callus induction (%)	Embryogenic callus induction (%)	No. of regenerated plants /100 anthers	
			Green	Albino
Line-A	1.16**	0.97**	0.24**	1.43**
Misr-1	-1.39**	-0.97**	-2.62**	-1.45**
Misr-2	-0.82**	-0.52**	0.05	0.22**
Gem-11*	1.05**	0.52**	2.32**	-0.20**
SE (gi)	0.09	0.36	0.08	0.06
F ₁ 's				
Line-A \times Misr-1	4.78**	1.75**	2.05**	3.24**
Line-A \times Misr-2	-2.95**	-1.70**	0.80**	-0.26**
Line-A \times Gem-11	-2.16**	-1.03**	-3.55**	-0.59**
Misr-1 \times Misr-2	-0.07	0.26**	-3.34**	-0.97**
Misr-1 \times Gem-11	-0.62**	0.19**	0.72**	0.198
Misr-2 \times Gem-11	5.57**	2.46**	5.47**	0.36**
SE (sij)	0.17	0.67	0.14	0.12
Reciprocals				
Misr-1 \times Line-A	4.08**	0.79**	1.17**	1.58**
Misr-2 \times Line-A	0.42*	-0.58**	-0.58**	1.75**
Gem-11 \times Line-A	-0.75**	-0.17*	-2.00**	-0.67**
Misr-2 \times Misr-1	-1.92**	-1.81**	0.75**	-0.33*
Gem-11 \times Misr-1	-0.92**	-0.33**	-2.25**	1.25**
Gem-11 \times Misr-2	4.17**	2.28**	-2.17**	-0.75**
SE (sij)	0.21	0.85	0.18	0.15

*and ** significant and high significant at 0.05 and 0.01 probability levels, respectively.

Present study also revealed that there were no significant reciprocal effects in the two crosses; Line-A \times Misr-1 and Line-A \times Gemmeiza-11 compared with their reciprocal crosses for all studied traits. On the contrary, the reciprocal effects were detected in the two crosses; Line-A \times Misr-2 and Misr-2 \times Gemmeiza-11 for green and albino plant regeneration as well as the two crosses; Misr-1 \times Misr-2 and Misr-1 \times Gemmeiza-11 for embryogenic callus and green plant regeneration. Many evidences illustrate that genetic factors, mostly nuclear genes are major factors for determination of anther culture response. It is also known that the external factors influence the anther culture response as explained previously (Massiah *et al.*, 2001). Although genetic differences within genotypes reveal physical and biochemical differences, molecular events that trigger anther

culture response should be studied in the future (Dagustu, 2008).

In conclusion, the results indicated that preponderance of non-additive gene action (dominance and epistasis) in the inheritance of all studied traits. Percentage of genetic contribution calculated for all studied traits are presented in Fig. (1). The SCA variance had the highest percentage of genetic contribution for all studied traits; callus induction (58.34), embryogenic callus induction (48.28), number of green plants (50.77) and number of albino plants (42.12), followed by reciprocal variance for callus induction (27.53) and then the GCA variance for number of green plants (37.13) and number of albino plants (38.87).

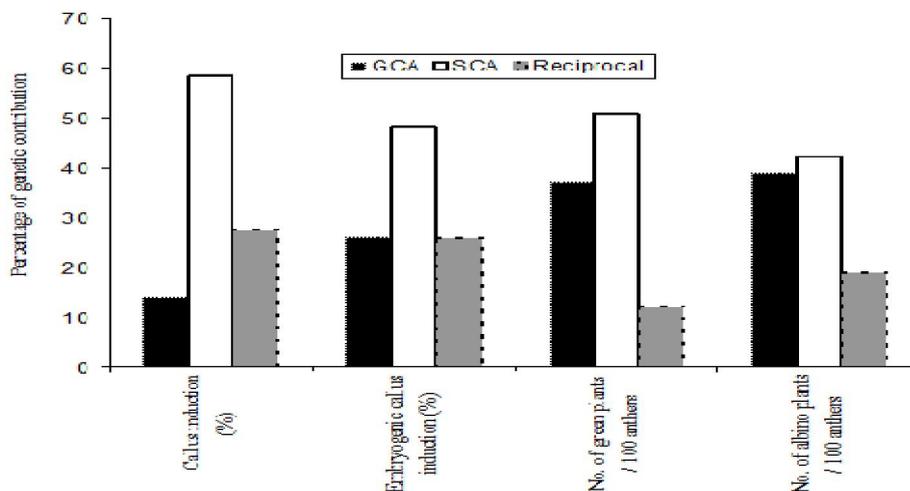


Fig. 1: Percentage of genetic contribution for the androgenic response of wheat F₁ hybrids.

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