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GENETICAL AND CYTOLOGICAL STUDIES ON BREAD WHEAT ANTHER CULTURE.*

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Abstract: Genetical and cytological studies were carried out on anther culture response of five hexaploid wheat genotypes (*Triticum aestivum L*). Five different callus induction media were used to study the effect of callus induction medium on anther culture response. The NPB98-medium induced the highest % of anthers produced calli and calli developed from such medium also regenerated the highest No. of green plants/100 anther, No. of albino plants/100 anther and % of green/total regenerated plants. Such medium was suitable for wheat anther culture. The genotype ICR-33 followed by ICR-DH displayed the highest androgenic response than the other genotypes. Meanwhile, Gimmeiza-7 was the lowest responsive one however, Giza-164 showed low androgenic response. Approximately no green plants were regenerated from the genotype Selection 1x15. Callus formation, number of regenerated green and albino plants are controlled by different genes leading to independent inheritance of these parameters. Most of the examined androgenic plants (91.92%) were haploid (1n = 3X = 21 chromosomes) while, only 8.08% were hexaploids (2n = 6X = 42). However, the percentages of ploidy levels differed from one genotype to another as well as between green and albino plants. In all tested genotypes, the albino plants showed higher percentages of haploids as compared to the green plants. The A-medium followed by NPB98-medium enhanced regeneration of the haploid plants as compared to the P4-medium. Out of 332 haploid plants treated with colchicine for chromosome doubling, 36 from ICR-DH, 75 from ICR-33 and 13 from Giza-164 were established in the field and only five of these plants produced seeds. This investigation suggested that the establishment of anther culture method in bread wheat offers the opportunity of obtaining haploid and/or doubled haploid plants which could be used for wheat improvement.

Keywords: Wheat, anther culture, cytology

Introduction

Bread Wheat (*Triticum aestivum* L.) is considered as the essential strategic cereal crop not only in Egypt but also allover the world. It is an hexaploid species and a

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member of the family *Gramineae*. The production of wheat needs to be increased annually to maintain supply for the increasing consumer population. This fact motivates biotechnologists to search new approaches such as cell, anther and tissue culture in addition to genetic engineering, which develop the breeding methods to improve the genetic characters in such crop.

Anther culture technique has attracted considerable attention as supplementary tools to cereal crop improvement (Vasil and Vasil, 1994). Anther culture involves the induction of embryoid formation from immature pollen and subsequent regeneration of embryoids into plantlets. Anther culture-derived haploids have been used to produce complete homozygous lines, which accelerate breeding programs, easy isolation of recessive mutants, selection of phenotypes for quantitative characters, and fix agronomic traits in the homozygous state (Kasha *et al.*, 1990). It is also useful in mutation breeding, *in vitro* selection, and transformation techniques (Kasha and Maluszynski, 2003). Despite the progress made since the first attempts at wheat anther culture more than 30 years ago (Ouyang *et al.*, 1973; Picard and De Buyser, 1973), strong genotype dependence is still one of the major obstacles to its application. Moreover, success in the anther culture method depends on plant growth conditions, media composition, and handling of cultures (Ouyang *et al.*, 1983; Lazar *et al.*, 1984; De Buyser and Henry, 1986; Zhou and Konzak, 1989; Ziegler *et al.*, 1990; Ozgen *et al.*, 1998; Liu *et al.*, 2002 and Datta, 2005).

In general, *in vitro* wheat haploid production requires a multiple-step procedure (Zheng, 2003): (1) induction of microspore-derived embryos or calluses, (2a) plant regeneration from embryos (embryogenesis; Rybczynski *et al.*, 1991) or (2b) the initiation and growth of shoots followed by root development (organogenesis; Niizeki and Oono, 1968), and (3) chromosome doubling of regenerated plants, if necessary.

In the present investigation, genetical and cytological studies were carried out on anther culture response of five hexaploid wheat genotypes. Establishment of anther culture method in wheat offers the opportunity of obtaining haploid and/or doubled haploid plants which could be used for wheat improvement.

Materials and Methods

Plant Materials: Five genotypes of hexaploied wheat (*Triticum aestivum L*), namely; Giza 164 (Kvz / Buha "s" // Kal / Bb), Gimmeiza-7 (CMM 74A. 630 / Sx // Seri 82 / 3/ Agent),

Selection 1x15 (Advanced breeding line derived from inter population-interenvironmental cross between early segregates selected in two contrasting environments), ICR33 (ICARDA) and ICR-DH (ICARDA), were used in the present investigation.

Anther culture procedure:

The five donor parents were used to establish anther culture technique in wheat and to study the genetical and cytological studies on wheat anther culture.

Collecting tiller: Donor spikes were collected at the mid to late uninuclate stage. Suitable tiller could be recognized when the upper part of the spike had reached the upper one third of the flag leaf sheath. To verify that the microspores were at the mid to late uninuclate stage, one or two anther from a central flower of the spike were squashed in a drop of acetocarmine on a glass slid for microscopic test.

Pretreatment of spikes: All leaves except flag leaf were removed, the bases of collected tillers were put in glass beaker containing tap water and the top covered with aluminum foil to maintain high humidity and refrigerated at 4°C in darkness for 3-7 days

Sterilization and anther culture: The spikes were removed from the flag leaf sheath and sterilized. Sterilization was carried out under sterile condition in 100% ethanol for one minute and air dried in a sterile laminar flow bench. The anthers were aseptically excised by fine sterilized forceps and plated in Petri dishes containing callus induction medium. The dishes were sealed with parafilm and incubated at 28 ± 2°C for 8 weeks (seven weeks in darkness and one week in illumination).

Callus induction medium: In order to study the effect of callus induction medium on anther culture response in wheat, five different callus induction media were used. The five media types are P2-medium (P2 basic salts + 1.5mg/l 2,4-D + 0.5mg/l kinetin + 10% potato extract + 90.0g/l sucrose + 8g/l agar, Ouyang *et al.*, 1978), P4-medium (P4 basic salts + 1.5mg/l 2,4-D + 0.5mg/l kinetin + 10% potato extract + 90.0g/l sucrose + 8g/l agar, Ouyang *et al.*, 1983), C17-medium (C17 basic salts + 2.0mg/l 2,4-D + 0.5mg/l kinetin + 90.0g/l sucrose + 8g/l agar, Wang and Chen, 1986), A-liquid medium (A basic salts + 1.5mg/l 2,4-D + 0.5mg/l kinetin + 10% potato extract + 200mg/l glutamin + 90.0g/l sucrose, Zhang *et al.*, 1987), and NPB98-liquid medium (NPB98 basic salts + 0.2mg/l 2,4-D + 0.2mg/l kinetin + 0.1mg/l PAA + 500mg/l glutamin + 90.0g/l sucrose + 90.0g/l maltose, Liu *et al.*, 2002). The pH for all media was adjusted to be 5.8 and

autoclaved (121°C for 20 minutes) at 1.2 Kg cm^2.

Plant regeneration: The developed calli (approx. 1 - 2 mm in diameter) were transferred to "190-2" regeneration medium (190-2 basic salts + 0.5mg/l kinetin + 0.5mg/l NAA + 30.0g/l sucrose, pH 5.8, Zhuang and Jia, 1983). The cultures were incubated at 21± 2°C with 16 hours photoperiod (1000 – 1500 LUX) for 4 weeks.

The experiment was designed as a randomized complete block with three replications; each replication consisted of 125 Petri dish's (five Petri dishes per genotype and per callus induction medium). Each Petri dish containing about 80-100 anthers. The % of responded anthers (callus formation), No. of green plants per 100 anthers, No. of albino plants per 100 anthers, frequency of green plants per total regenerated plants were determined.

Analysis of variance was used to examine the significance of differences between the genotypes, media effect and their interaction on % of responsive anthers, No. of green and albino plants, and the percentage of green plants per total regenerated plants. LSD was used to examine the differences between means. Data analysis was performed by MSTAT-C (1990) computer statistical analysis program.

Transplantation: The well-rooted plants were transplanted to pots contained 1:1 mixture of peat and sand. Pots were covered with a transparent plastic cover for two weeks to keep the humidity, after that the transparent plastic were removed.

Cytological analysis of regenerated plants:

Root tips were collected from albino and green regenerated plants after 1-2 weeks of transplantations. They were treated with 0.3% colchicines solution for 4 hours, and then fixed in fresh mixture of glacial acetic acid and absolute ethanol 1: 3, V/V. The root tips were heated to boiling in 2.5% acetocarmen and squashed in a drop of acetic acid 45% under cover glass. Three root tips per plant and at least five cells per root tip were analyzed to determine mitotic chromosome numbers in the regenerated plants.

Chromosome doubling:

Four to five weeks old haploid plants were removed from pots and washed with running tap water, the plants were immersed in 0.3% colchicine solution for 4 hours at 20-23°C in dark. The plants were removed from colchicines solution and rinsed with running tap water overnight then repotted in pots and placed in the field up to harvesting.

Results and Discussion

Generally, the results revealed that calli from anthers containing mid-to-late uninucleate microspores grew to be visible after 5 to 8 weeks of incubation (Fig. 1). The incubation period depended upon the speed of initiation of calli of different genotypes. The developed calli with globular structure were transferred to 190-2 regeneration medium (Fig. 2). After 1 to 2 weeks the globular structure were then developed into shoots and roots (Fig. 3). The good developed plants (green and/or albino) were transferred to pots containing 1:1 mixture of peatmoss and sand. Regenerated 2 - 3 weeks plants (green and/or albino) were cytologically examined.

Anther culture responsiveness or androgenic competence can be separated into two major phases, the first where microspores are competent to develop into embryos or calli in anther culture and the second, where androgenic embryos or calli are competent to regenerate into plants. Since albinism is a common feature of microspore-derived plants in wheat, the regenerated plants were separated in the present investigation in three parameters, No. of green plants, No. of albino plants and frequency of green/total regenerated plants. The results are in conformity with earlier reports of De Buyser *et al.*, (1992) and Ozgen *et al.* (1998).

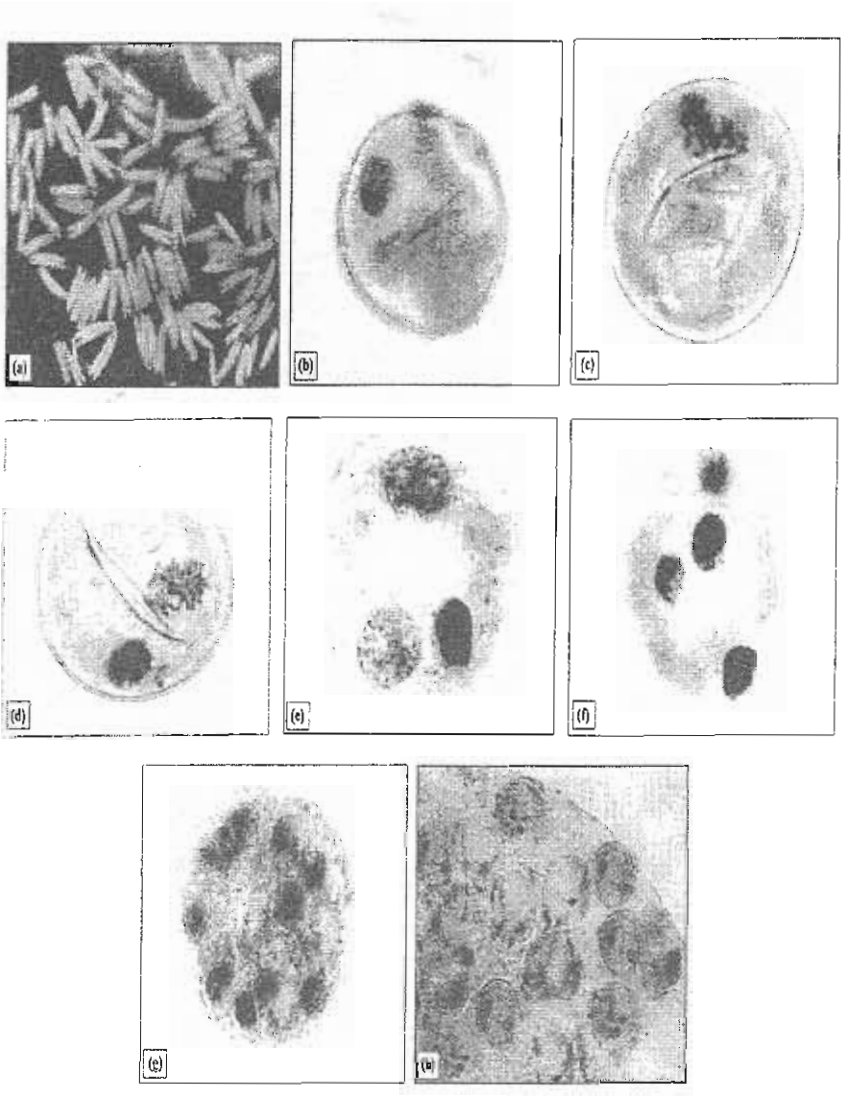
Overall genotypes, the results in Table (1) showed that the medium (NPB98) induced the highest % of anthers produced calli (42.83%). In addition, calli developed from such medium also regenerated the highest No. of green plants/100 anther (34.45), No. of albino plants/100 anther (27.06) and % of green/total regenerated plants (54.41%). Moderate % of anthers produced calli, No. of green plants, No. of albino plants/100 anther and % of green/total regenerated plants were developed on medium-A (23.36%, 11.71, 24.29 and 30.65%, respectively) and P4-medium (20.23%, 10.20, 11.94 and 43.74%, respectively). While, the P2 and C17 media displayed the lowest values of anther culture parameters. These differences between the tested media were highly significant (Table 2).

Since the frequency of green plants per total plants regenerated from anther culture reflects the efficiency of anther culture response in the tested genotypes, the medium NPB98, which displayed the highest % green/total plants, was much more effective for wheat anther culture.

The results in Table (1) showed that ICR-33 followed by ICR-DH displayed the highest

Table(1): The mean values of percentage of anthers produced calli, number of green plants/100 anthers, number of albino plants/100 anthers and percentage of green plants/total plants of the five wheat genotypes cultured on five callus induction media P2, P4, C17, A and NPB98 and regenerated on 190-2 medium.

Genotypes	Characters	Callus induction media					MEAN
		P2	P4	C17	A	NPB98	
ICR-DH	% of anthers produced calli	21.44	33.81	19.22	42.60	87.93	41.00
	No. of green plants/100 anthers	2.41	10.43	0.62	14.48	54.54	16.51
	No. of albino plants/100 anthers	10.02	14.45	3.99	32.14	44.08	20.94
	% of green plants/total plants	19.08	42.14	12.17	31.14	55.24	32.08
ICR-33	% of anthers produced calli	15.01	29.96	13.16	30.28	70.01	31.69
	No. of green plants/100 anthers	5.21	23.90	1.01	35.36	100.12	33.12
	No. of albino plants/100 anthers	15.67	16.92	4.56	34.02	22.75	18.79
	% of green plants/total plants	25.06	58.27	17.61	51.04	81.42	46.68
Selection 1x15	% of anthers produced calli	8.09	14.27	7.05	32.58	39.09	20.38
	No. of green plants/100 anthers	0.01	0.01	0.01	0.01	0.01	0.01
	No. of albino plants/100 anthers	4.69	15.75	0.83	36.89	58.89	23.41
	% of green plants/total plants	0.01	0.01	0.01	0.01	0.01	0.01
Giza-164	% of anthers produced calli	10.18	21.26	7.10	9.27	15.43	12.65
	No. of green plants/100 anthers	1.73	14.20	0.21	4.83	11.89	6.57
	No. of albino plants/100 anthers	9.66	11.07	1.97	13.74	7.60	8.81
	% of green plants/total plants	15.06	56.25	8.90	25.92	60.75	33.38
Gimmeiza-7	% of anthers produced calli	0.57	1.86	0.01	2.05	1.68	1.23
	No. of green plants/100 anthers	0.16	2.46	0.01	3.82	5.67	2.42
	No. of albino plants/100 anthers	0.81	1.53	0.01	4.59	1.98	1.79
	% of green plants/total plants	10.55	62.04	0.01	45.13	74.61	38.47
MEAN	% of anthers produced calli	11.22	20.23	9.31	23.36	42.83	
	No. of green plants/100 anthers	1.91	10.20	0.37	11.71	34.45	
	No. of albino plants/100 anthers	8.17	11.94	2.27	24.29	27.06	
	% of green plants/total plants	13.95	43.74	7.87	30.65	54.41	



Fig(1): Wheat anther culture: (a): anthers of wheat cultured on callus induction medium. (b): uninucleate microspore at the time of anther culture. (c): wheat microspore at the first mitotic division (metaphase) after 1 – 2 days of culture. (d): Binucleate microspores with one dividing nucleus. (e and f): multi-cellular microspores. (g): multi-cellular mass breaking out of microspore wall. (h): anthers with microspores at different developmental stages.

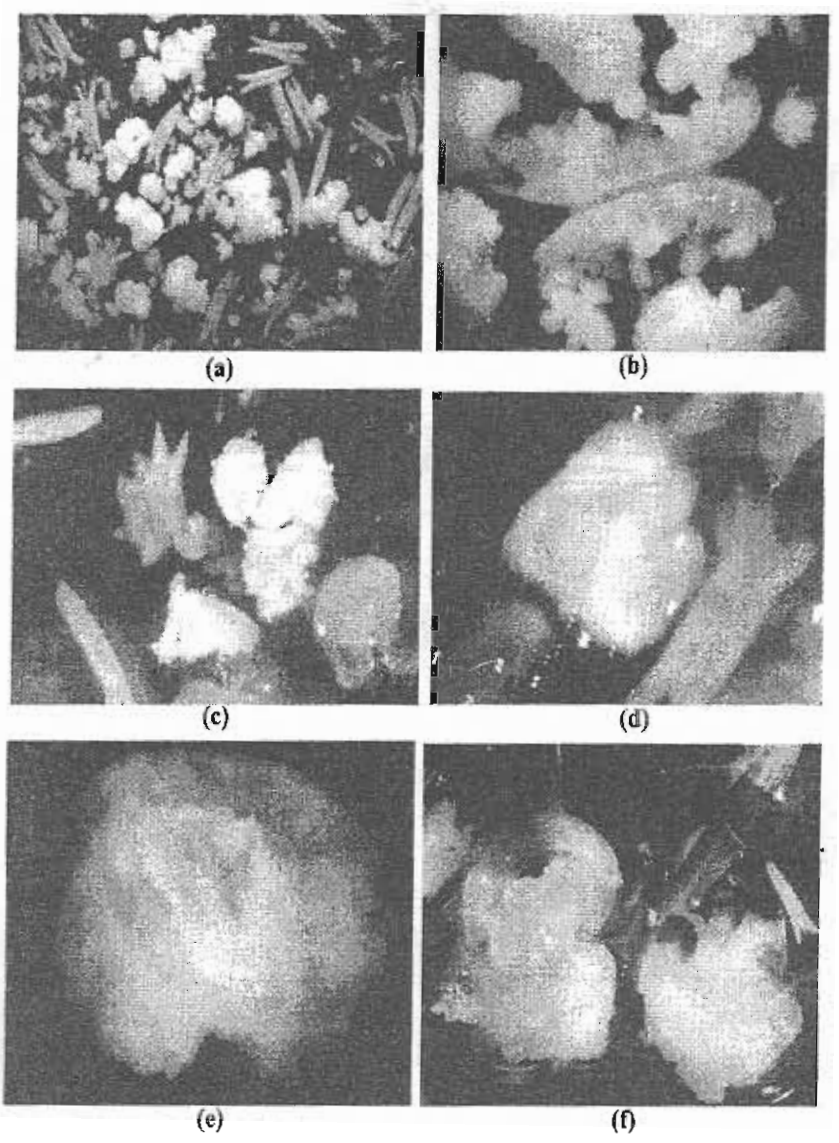


Fig.(2): The development of androgenic embryos from wheat anther culture: (a): Eight weeks old wheat anther culture (seven weeks in darkness and one week in illumination). (b): A highly responsive anther with calli, pro-embryoids and embryo just breaking through the anther wall. (c and d): Typical anther culture derived embryos. (e and f): Regenerating shoot (e) and germinating embryos from embryogenic callus (f).

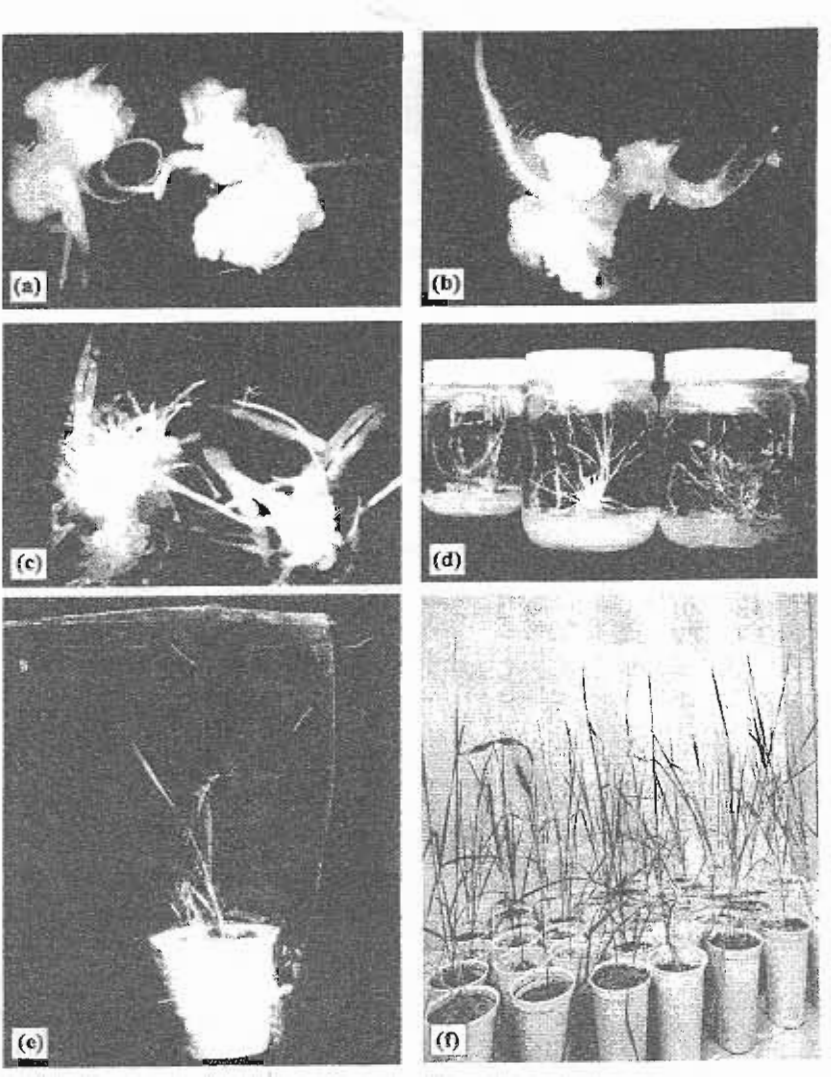


Fig.(3): The development of wheat plants from anther culture: (a): Germinating embryos with developed shoots (albino and green) and root. (b): Further development of the microspore derived plantlet. (c): Multiple shoots (green and albino) and roots. (d): Further growth of the regenerated green and albino plants in bottles. (e): Well rooted androgenic plant transplanted to pot and covered with transparent plastic bag for hardening. (f): successfully transplanted anther culture-derived wheat plants, some of them flowered and produced spikes in the growth chamber.

androgenic response than the other genotypes. They revealed the highest % of responsive anthers (31.69 and 41.00%, respectively) and No. of green plants (33.12 and 16.51 plants, respectively). The % of responsive anthers and No. of green plants were 20.38% and 0.01 in Selection 1x15, 12.65% and 6.57 in Giza-164, 1.23% and 2.42 in Gimmeiza-7, respectively. Approximately no green plants were regenerated from the genotype Selection 1x15. These differences between the genotypes were highly significant (Table 2). Liu *et al.* (2002) found that the % of green plants regenerated from wheat microspores ranged from 8 – 99% depending on the genotype. Bruins and Snijders (1995) found that the regeneration frequency was 12.8 plants per 100 wheat anthers, of which 25.6% was green, whereas Tuvesson *et al.* (1989) reported a percentage of 23.4 plants per 100 plated anthers of which 15.3% was green. However, Ouyang *et al.* (1983) produced 72 green plants per 100 anthers in wheat cultivar "Ciano 067". Hassawi *et al.* (2005) found that green plantlet regeneration was influenced significantly by cultivar and the highest frequency was produced by cultivar Acsad-65. Albino plantlets were produced by cultivars Hourani Nawawi and Acsad 65.

High significant interactions between genotypes and callus induction media were observed in all characters of anther culture response suggesting the dependence of anther culture response in wheat on the genotype as well as the type of medium used for callus formation. Table (1) revealed that changing the medium components could greatly change the parameters of anther culture response in the high responsive genotypes ICR-33 and ICR-DH. Meanwhile, none of the tested media could improve anther culture response in the lowest responsive genotype GM-7. These results confirmed the dependence of anther culture response in wheat mainly on the genotype and subsequently on the type of induction medium.

Non-significant correlations were observed between callus formation and each of number of regenerated green ($r = 0.6619$) and albino ($r= 0.8207$) plants, as well as between the regeneration of green and albino plants ($r = 0.3361$), which suggested that they are controlled by different genes leading to independent inheritance of these parameters. These results also suggested that anther culture response in wheat is a heritable and complex character involving at least two different and separately inherited mechanisms: the ability of microspores within anthers to divide and give rise to callus, and

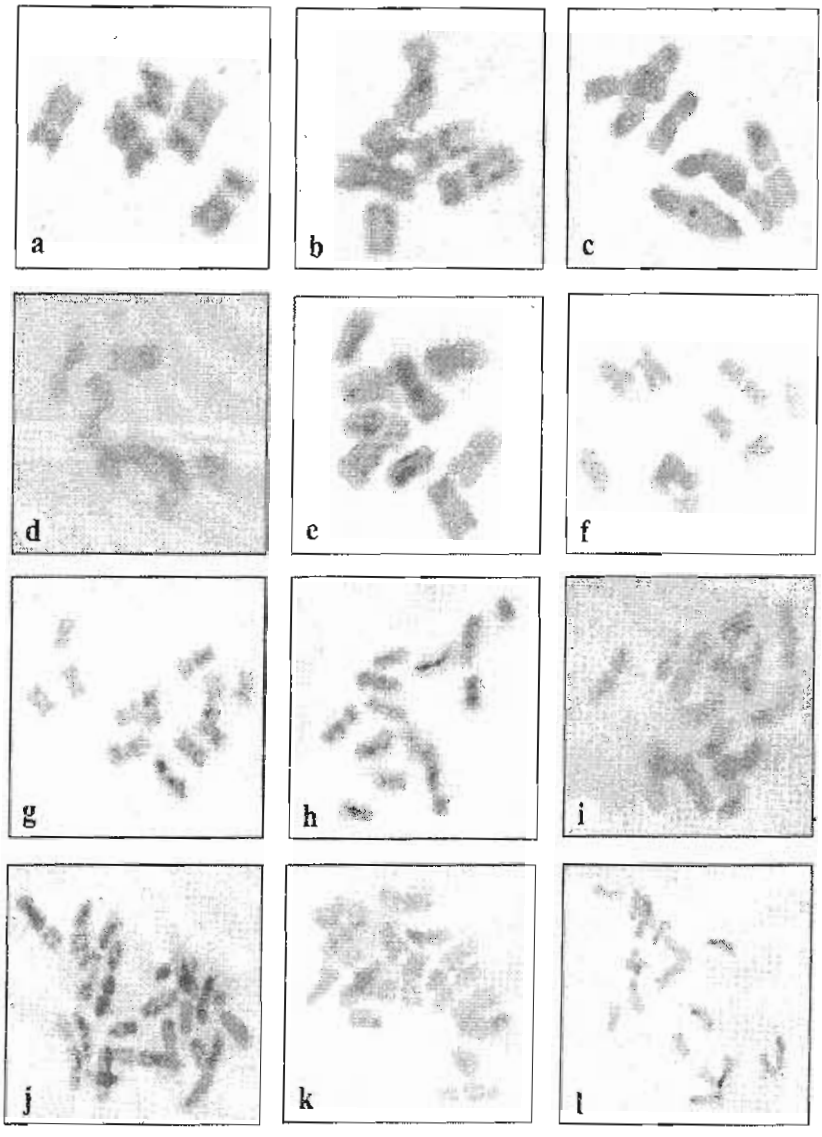
the ability of callus for morphogenesis to yield green or albino plants. Ozgen *et al.* (1998) found insignificant correlations between callus induction rate, weight of callus and regeneration capacity in both mature and immature embryo cultures of wheat, suggesting that these culture traits are independent from each other and likely to be controlled by different genes. Similar results were also obtained by De Buyser *et al.*, (1992) and Ekiz and Konzak (1994).

Albinism is a common feature of microspore-derived plants, however the formation of albino plants is a serious problem in the anther culture of cereals. In the present study, albino plants were regenerated from all tested genotypes in different frequencies and approximately all plants regenerated from the genotype Selection 1x15 were albino. In hexaploid wheat, it has been demonstrated that the ratio of green to albino plants obtained is strongly affected by genes in the parental material (Andersen *et al.*, 1987). The occurrence of albino plants has been found to be 97% for wheat cultivar 'Edwall' (Zhou and Konzak, 1989) and 88% averaged over four German spring wheat cultivars (Ziegler *et al.* (1990). Genetic background of the donor plants is an important factor affecting the extent of albinism while cold pretreatment in general and the use of Ficol as a floating agent in the liquid medium may delay or arrest nuclear synchronization and help in producing green plants (Datta, 2005). The regeneration of green and albino plants seems to be affected by different genes localized on different chromosomes (De Buyser *et al.*, 1992) and may also affected by the environment (Opsahl-Ferstad *et al.*, 1994). This is consistent with the general concept of albinism, as being sustained by mutations induced by the *in vitro* conditions in addition to the effect of nuclear genes. In wheat, many studies reported that albinism was caused by a deletion in chloroplast genome (Harada *et al.*, 1991; Zubko and Day, 2002 and Mozgova *et al.*, 2006).

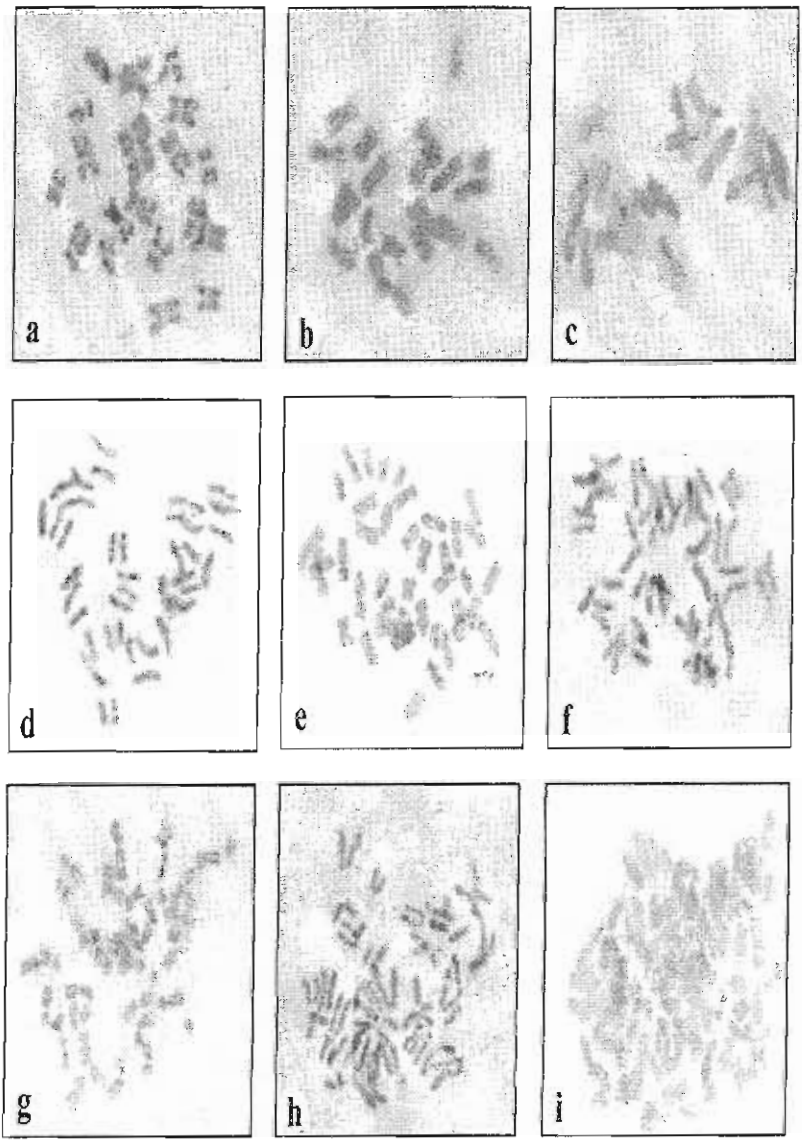
Cytological analysis:

a) Ploidy level of regenerated plants:

Ploidy level of a total of 396 R_0 plants (238 green and 158 albino) derived from anther culture of the genotypes ICR-DH, ICR-33 and Giza-164 was determined by chromosome counts in root tip cells (Figs.4 and 5) and summarized in Table (3). These plants are distributed as, 155 (100 green and 55 albino) from ICR-DH, 174 (106 green and 68 albino) from ICR-33 and 67 (32 green and 35 albino) from Giza-164.



Fig(4): Chromosomal variations observed in root tip cells of wheat plants regenerated from anther culture where: (a) 5 chromosomes, (b) 6 chromosomes, (c) 7 chromosomes, (d) 8 chromosomes, (e) 9 chromosomes, (f) 11 chromosomes, (g) 12 chromosomes, (h) 14 chromosomes, (i) 16 chromosomes, (j) 17 chromosomes, (k) 18 chromosomes and (l) 19 chromosomes.



Fig(5): Chromosomal variations observed in root tip cells of wheat plants regenerated from anther culture where: (a) 20 chromosomes, (b) 21 chromosomes, (c) 23 chromosomes, (d) 38 chromosomes, (e) 42 chromosomes, (f), (g), (h) and (i) > 42 chromosomes

Table(2): The analyses of variance for androgenic response traits in five wheat genotypes in relation to 5 types of callus induction media.

Source of variation	d.f	% of responsive anthers	No. of Green plants/100 anthers	No. of albino plants/100 anthers	% of green plants/total regenerated plants
Replicates	2	10.071	4.816	7.495	12.41
Genotypes (G)	4	2678.056**	2789.9**	1684.77**	5745.723**
Media (M)	4	3653.547**	2740.702**	1248.579**	4743.843**
G x M	16	490.667**	897.105**	333.226**	490.835**
Error	48	7.001	6.469	3.85	7.398

Table(3): Number and frequency of the ploidy level in albino and green plants regenerated from anther culture of the genotypes ICR-DH, ICR-33 and Giza-164.

Genotypes	Regenerated plants	No of regenerated plants	Haploid		Hexaploid	
			No	%	No	%
ICR-DH	Albino	55	54	98.18	1	1.82
	Green	100	93	93.00	7	7.0
	Total	155	147	94.84	8	5.16
ICR-33	Albino	68	64	94.12	4	5.88
	Green	106	96	90.57	10	9.43
	Total	174	160	91.95	14	8.05
G-164	Albino	35	32	91.43	3	8.57
	Green	32	25	78.13	7	21.88
	Total	67	57	85.07	10	14.92
Grand total	Albino	158	150	94.94	8	5.06
	Green	238	214	89.92	24	10.08
	Total	396	364	91.92	32	8.08

Generally, most of the examined plants (364 plants, 91.92%) showed the expected haploid chromosome number (1n = 3X = 21) in most tested cells of root tips (Table 3, Fig. 5). Only 32 (8.08%) androgenic plants revealed the hexaploid chromosome number (2n = 6X = 42) in the examined cells. However, the percentages of ploidy levels differed from one genotype to another as well as between green and albino plants (Table 3). The frequency of spontaneous diploids may differ due to several factors, such as anther culture media (Henry and De Buyser, 1990), culture age and culture temperature (Ahmed *et al.*, 1997), cold pretreatment (Amssa *et al.*, 1980) and genotype (Ouyang *et al.*, 1994).

Over all green and albino plants, the results in Table (3) revealed that the genotype ICR-DH showed higher percentage of haploid plants (94.84%) than ICR-33 (91.95%) and G-164 (85.07%). In contrast, the highest % of hexaploids was observed in plants regenerated from Giza-164 (14.92%) as compared with ICR-33 (8.05%) and ICR-DH (5.16%). **In wheat anther culture, Liang** et al. (1982) found that the regenerated plants were predominantly haploids ($1n = 3x = 21$). Different studies reported that spontaneous doubling frequencies of plants regenerated via anther cultures from wheat ranged from 5 to 30% (Masojc *et al.*, 1993 and Navarro-Alvarez *et al.*, 1994).

Variation in chromosome number was also observed between green and albino plants. Out of the three genotypes, a total of 238 green and 158 albino plants were tested for ploidy level (Table 3). In all tested genotypes, the albino plants showed higher percentages of haploids and lower percentages of hexaploids as compared to the green plants. On average, the percentage of haploids and hexaploids were 94.94% and 5.06% in albino plants and were 89.92% and 10.08% in green plants, respectively. However, these percentages differed from one genotype to another. Green or albino plants regenerated from Giza-164 possessed the lowest percentage of haploids and the highest percentage of hexaploids as compared to the other two genotypes.

In all plant regeneration experiments, the nature and the tissue origin of the regenerated progeny are of primary interest. While there is never much doubt that haploids from anther culture must originate from some products of meiosis. The origin of diploids (that is, plants with the same chromosome numbers as the somatic chromosome number of the donor) is never certain without additional tests. Such diploids can, at least in theory, regenerate from the somatic tissue of the anther being clones of the parental plants,

or originate from microspores subjected to spontaneous doubling under culture conditions which will be completely homozygous (Bante *et al.,* 1990). Identification of homozygous plants is of particular interest since these plants are equally valuable as the monoploid ones. The origin of the diploid progeny can be tested by segregation or genetic markers studies (Andersen *et al.,* 1997).

b) Effect of callus induction medium on the ploidy level of regenerated plants:

The effect of callus induction medium on the ploidy level of plants regenerated from ICR-DH, ICR-33 and Giza-164 was studied and summarized in Table (4). The results revealed considerable variations in chromosome number of the tested plants regenerated from different callus induction media. The androgenic plants developed from A-medium showed the highest percentage of haploids (96.46%) and lowest hexaploids (3.54%), as compared to those of other media. In contrast, the P4-medium regenerated the lowest percentage of haploids (82.76%) and highest hexaploids (17.24%). The NPB98-medium induced calli which developed into 93.37% haploid and 6.63% hexaploid plants. However these percentages differed from one genotype to another. In the genotype ICR-DH, the percentage of haploids and hexaploids were 98.00 and 2.00% on A-medium while they were 93.75 and 6.25% on NPB98-medium, respectively. The "NPB98" and "A" media showed a relatively similar effect on the production of haploid (94.44 and 93.48%, respectively) and hexaploid (5.56 and 6.52%, respectively) of the genotype ICR-33. In Giza-164, all plants developed from the A-medium were haploids while those from NPB98-medium showed 88.46% haploids and 11.54% hexaploids.

These results showed that the A-medium followed by NPB98-medium enhanced regeneration of the haploid plants as compared to the P4-medium. Since calli developed from the NPB98-medium regenerated the highest % of green plants (54.41%, Table 1), these results strengthen the previous suggestion that the medium NPB98 was much bettet for callus induction in wheat anther culture and regeneration of more haploid plants.

c) Variability of chromosome numbers in root-tip cells of the regenerated plants:

Chromosome counts were made in 5520 cells examined from root tips of 396 androgenic plants (Table 5, F ig. 4and 5). These examind cells are distributed as: 2202 (1414 green and 788 albino) were from ICR-DH, 2349 (1430 green and 919 albino) from ICR-33 and 969 (460 green and 509 albino) from Giza-164 (Table 5).

Table(4): Effect of callus induction medium on the frequency of ploidy level of the androgenic plants regenerated from anther culture of the genotypes ICR-DH, ICR-33 and Giza-164.

Genotypes	Callus induction media	No of regenerated plant	Haploid		Hexaploid	
			No	%	No	%
ICR-DH	NPB98	80	75	93.75	5	6.25
	P4	25	23	92.00	2	8.00
	A	50	49	98.00	1	2.00
ICR-33	NPB98	90	85	94.44	5	5.56
	P4	38	32	84.21	6	15.79
	A	46	43	93.48	3	6.52
G-164	NPB98	26	23	88.46	3	11.54
	P4	24	17	70.83	7	29.17
	A	17	17	100.00	0	0.00
Total	NPB98	196	183	93.37	13	6.63
	P4	87	72	82.76	15	17.24
	A	113	109	96.46	4	3.54

Table(5): Variability of chromosome numbers in root-tip cells of green and albino plants regenerated from anther culture of the genotypes ICR-DH, ICR-33 and Giza-164.

Genotypes	Regenerated plants	No. of regenerated plants	No. of examined cells	% of cells having chromosome number				
				<21	21	22-41	42	>42
ICR-DH	Albino	55	788	24.08	67.49	4.68	3.75	0.0
	Green	100	1414	21.43	66.43	8.27	3.11	0.76
	Total	155	2202	22.75	66.96	6.48	3.43	0.38
ICR-33	Albino	68	919	18.91	70.42	4.63	4.18	1.86
	Green	106	1430	14.79	71.63	5.58	7.78	0.22
	Total	174	2349	16.85	71.03	5.11	5.97	1.04
G-164	Albino	35	509	13.04	70.33	6.42	5.30	4.91
	Green	32	460	11.33	62.96	8.67	17.04	0.0
	Total	67	969	12.18	66.65	7.54	11.17	2.46
Grand total	Albino	158	2216	18.67	69.42	5.24	4.41	2.26
	Green	238	3304	15.85	67.01	7.51	9.31	0.32
	Total	396	5520	17.26	68.21	6.38	6.86	1.29

Generally, the mitotic counts revealed considerable variations in chromosome numbers of the examined cells ranged from less than 21 to higher than 42, with a majority being in the haploids with 21 (68.21%) chromosomes (Table 5 and Figs. 4 and 5). Average over the genotypes, the hexaploids (42 chromosomes) were represented by 6.86% of the examined cells. A number of the examined cells showed aneuploidy, represented by 17.26% (< 21 chromosomes), 6.38% (22 – 41 chromosomes) and 1.29% (more than 42 chromosomes) of the examined cells (Table 5). These results varied from one genotype to another as well as between green and albino plants.

Average over green and albino plants, ICR-33 showed higher percentage of haploid cells (71.03%) than ICR-DH (66.96%) and G-164 (66.65%). While, the highest % of hexaploids was observed in root cells of Giza-164 (11.17%) as compared with ICR-DH (3.43%) and ICR-33 (5.97%). However, the highest % of aneuploids was observed in root cells of ICR-DH for <21 chromosomes (22.75%) and in Giza-164 for 22-41 chromosomes (7.54%) and for >42 chromosomes (2.46%).

Variation in chromosome number in root-tip cells was also observed between green and albino plants. Average over the genotypes, the % of haploid cells was higher in albino plants (69.42%) than green ones (67.01%) while the % of hexaploid cells was greater in green plants (9.31%) than that in albino plants (4.41%) (Table 5). The % of aneuploid cells having chromosome numbers less than 21 or more than 42 were higher in albino plants (18.67% and 2.26%) than green plants (15.85% and 0.32%). In contrast, the green plants showed higher percentage of aneuploid cells with 22-41 chromosomes (7.51%) than root-tip cells of albino plants (5.24%). These results were observed in all tested genotypes with some exceptions. In ICR-DH, the root cells of the green plants showed lower percentage of hexaploids (3.11%) and higher % of >42 aneuploids (0.76%) than albino cells (3.75%, 0.00%). The root cells of green plants of ICR-33 possessed a relatively high % of haploids (71.63%) than root cells of albino plants (70.42%).

Aneuploidy may result from translocation, mitotic non-disjunction, or deletions (Evans and Reed, 1981). It was found that several factors can strongly affect the genetic stability in haploid cell cultures e.g. genotype of donor plants, culture medium and callus induction conditions (Henry and De Buyser, 1990 and Ziauddian and Kasha, 1990). Aneuploids, such as nullisomics, monosomics,

tetrasomics, various heteroploids (e.g. 35 chromosomes), as well as abnormal chromosome structures (e.g. telocentric lines) were observed from wheat anther cultures (Metz *et al.,* 1988; Feng and Ouyang, 1989).

Chromosome doubling and establishment of the regenerated plants:

For chromosome doubling, the 4-5 weeks old haploid plants (21 chromosomes) were treated with colchicines, then transferred to pots in the experimental lab and finally to field up to harvesting. A total of 489 haploid plants from ICR-DH (188 plants), ICR-33 (240 plants) and Giza164 (61 plants) were treated with 0.3% colchicines (Table 6). The % of viable plants after treatment ranged from 55.7% in Giza-164 to 74.2% in ICR-33 with an average of 67.9%. The numbers of plants established in the field were 36 from ICR-DH, 75 from ICR-33 and 13 from Giza-164. Only five of these plants (3 from ICR-33 and 2 from Giza-164) produced seeds.

Spontaneous rates of chromosome doubling among plants derived from microspores of wheat are relatively low. Colchicine is the most frequently used drug for chromosome doubling in plants. It inhibits spindle function during mitosis and disturbs normal polar segregation of sister chromatids to form a restitution nucleus. Upon mitotic divisions of such affected cells chromosome doubled chimeral sectors are formed, which lead to partial fertility of the plant if they comprise sexual organs. Inagaki, (1985) obtained dihaploid plants through treating the androgenic wheat plants at the 3-4 tillering stage with colchicines.

Table(6): Number of plants treated with colchicine, maintained in the lab., established in the field and produced seeds.

Genotypes	No. of treated plants with colchicine	No. of viable plants after treatment	No. of plants maintained in the lab.	No. of plants established in field	Plants Produced seeds
ICR-DH	188	120 (63.8%)	84	36	0
ICR-33	240	178 (74.2%)	103	75	3
G-164	61	34 (55.7%)	21	13	2
Total	489	332 (67.9%)	208	124	5

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دراسات وراثية وسيتولوجية على زراعة المتك في قمح الخبز.*

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تم إجراء دراسات وراثية وسيتولوجيه على الاستجابة لزراعة المتك باستخدام خمس تراكيب وراثية من قمح الخبز. استخدمت خمس بيئات مختلفة لتكوين الكالوس لدراسة تأثيرها على الاستجابة لزراعة المتك وقد أظهرت البيئة المغذية NPB98 أعلى نسبة لتكوين الكالوس من مزارع المتك والتي تكشف عنها أكبر عدد من النباتات الخضراء ونباتات الألبينو وأعلى نسبة من النباتات الخضراء بالنسبة للعدد الكلي للنباتات الناتجة ، لذا كانت هذه البيئة أكثر مناسبة لزراعة متك القمح مقارنة بالبيئات الأخرى وقد أظهر التركيب الوراثي ICR-33 يليه التركيب الوراثي ICR-DH أعلى استجابة لزراعة المتك بالمقارنه بالتراكيب الوراثية الأخرى بينما كان التركيب الوراثي جميزة-7 أقلهم استجابة في الوقت الذي أظهرفيه التركيب الوراثي جيزة-164 استجابة ضعيفة. ولم يتكشف عن التركيب الوراثي منتخب 1 15x أية نباتات خضراء حيث كانت النباتات المتكشفة منه البينو. و أوضحت النتائج أن كل من تكوين الكالوس وعدد النباتات الخضراء وعدد النباتات الألبينو تقع تحت تحكم جينات مختلفة يعتمد عليها نظام توارث هذه المقاييس.

وكانت معظم النباتات المختبره الناتجة من مزارع المتك أحادية بنسبة 91.92% (21 كروموسوم) بينما كانت النباتات الثنائيه بنسبة 8.08% (42 كروموسوم). وقد إختلفت نسب مستويات التضاعف من تركيب وراثي لآخر كما اختلفت هذه النسب بين النباتات الخضراء والألبينو. وأظهرت النباتات الألبينو أعلى نسب من النباتات الأحادية مقارنة بالنباتات الخضراء في جميع التراكيب الوراثية المختبرةحيث أعطت البيئة المغذية A يليها البيئه NPB98 نسب عالية من النباتات الأحادية مقارنة بالبيئه P4. ووجد أنه من بين 332 نبات أحادي تم معاملتها بالكولشسين لإجراء التضاعف الكروموسومي 36 نبات من التركيب الوراثي ICR-DH و 75 من ICR-33 و13من جيزة-164 أمكن توطيدهافي المزرعة حيث أنتجت خمسة نباتات فقط منها حبوب. و يقترح هذا البحث أن الطريقه المناسبة لزراعة المتك في قمح الخبز للحصول على نباتات أحادية أو سداسية أصيلة يمكن استخدامها في برامج التحسين الوراثي في القمح.

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