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Use of tissue culture to bypass wheat hybrid necrosis

Z. Chen [1], D. A. Evans [1] and A. Vasconcelos [2]

[1] DNA Plant Technology Corporation, 2611 Branch Pike, Cinnaminson, New Jersey, USA
[2] Department of Biological Sciences, Rutgers University, Newbrunswick, New Jersey, USA

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Summary. Hybrid necrosis in wheat is a barrier to gene transfer in wheat breeding practice. It is based on two complementary genes, Ne1 and Ne2. Recovery mutants (Re1, Re2 and Re3) which can grow to maturity were recovered from immature embryo cultures of necrotic hybrids between *T. aestivum* and *T. durum*. Cytological observation demonstrated that Re1 had 34 chromosomes instead of 35. This indicated that one of the chromosomes carrying the Ne genes was lost. Genetic study suggested that for Re1, the lost chromosome was chromosome 5B of the durum parental line. Re mutants are male sterile but can be maintained through a young ear culture method. Re mutants could be successfully pollinated by either parental line and the BC1 progeny is partially fertile. Re mutants were repeatedly induced in about 1% of the regenerated plants from immature embryo culture. This technique provides a practical way to bypass hybrid necrosis.

Key words: Hybrid necrosis – Wheat – Immature embryo culture – Young ear culture

Introduction

Hybrid necrosis is the premature gradual death of leaves and leaf sheaths in certain wheat hybrids. It is based on two complementary genes, Ne1 and Ne2 (Hermsen 1963a). If a Ne1 carrier is crossed with a Ne2 carrier (Ne1Ne1 × Ne2Ne2 or Ne2Ne2 × Ne1Ne1), the hybrid (Ne1ne1Ne2ne2) will express the necrotic syndrome. The degree of necrosis in the F_1 progeny varies greatly due to multiple allelism of Ne1 and Ne2 (Hermsen 1960). Ne^s, Ne^m and Ne^w are used for the symbols of strong, moderate and weak necrotic genes, respectively (Hermsen 1963b; Zeven 1966). In a survey of 4,629 wheat varieties tested, 28.0% are Ne1 carriers, 22.3% are Ne2 carriers and 49.7% are noncarriers (Zeven 1976). Intensive research on wheat necrosis and long listings of Ne carriers have been published since the mid-1960s (Zeven 1965, 1968, 1969, 1971, 1973, 1976, 1981; Kochumadhavan et al. 1980).

Nishikawa (1974) and Nishikawa et al. (1974) determined that Ne1 is located on the long arm of chromosome 5B and Ne2 on the short arm of chromosome 2B.

In general, avoiding the use of Ne carriers is the best way to deal with the necrosis problem in a wheat breeding program. However, this has not always been possible in practice. A second option (Hermsen 1963a) is to use a 'bridge variety' which has been documented to be a non-carrier. The bridge variety is first crossed with one of the parental lines with which hybridization is intended and desired lines are selected. The selected lines are used in a second cycle to cross with the second parental line, at which stage another selection must be completed. Obviously this is a complicated and time-consuming method and is not practical for screening a large number of crosses.

It is known that Ne^s1 is extremely common (Zeven 1976; Gregory 1980) in durum wheat (*Triticum durum*). Therefore, necrosis is especially serious when durum wheat is used as one of the parents in an interspecific cross. In this research, we use *T. aestivum* × *T. durum* as an interspecific crossing system and report a method that can be used to bypass the necrosis problem.

Materials and methods

Materials

Two bread wheat (*Triticum aestivum*) lines (CMB-2, CMB-6) and three durum wheat (*Triticum durum*) lines (CMD-1, CMD-7

and CMD-8) were used in the experiment. All the lines came from CIMMYT and were provided by Dr. N. E. Borlaug.

Materials were grown in a greenhouse, each in a 1-gal pot. They were watered routinely and no fertilizer was used.

Immature embryo culture

Immature embryos were harvested about 14 days after anthesis. Embryos about 1–2 mm (He et al. 1986) in size were placed on solid medium with the scutellum in contact with the culture medium. The callus induction medium was a modified MS medium with 2% sucrose and 1.5–2 mg/l 2,4-D. Three weeks after embryos were placed in culture, the induced calli were transferred to differentiation medium that contained MS macroelements at ½ strength, 0.1–15 mg/l 2,4-D and 0.5 NAA mg/l, while the kinetin content was adjusted in a range of 1.0 mg/l–5.0 mg/l according to the growth status of the calli. Cultures were transferred to new differentiation medium every 20–25 days until green leaf primordia appeared. Finally, the cultures were placed in ½ strength MS medium with 0.5 mg/l NAA for rooting.

Young ear culture

Young ears 1.5–3.5 cm in length which were at a stage prior to meiosis were cut into 2–5 pieces and cultured on induction medium. Two induction media were used. Medium A: MS basic components with 2% sucrose and 8–10 mg/l 2,4-D. Medium B: MS basic components with 2% sucrose and 2 mg/l 2,4-D. If medium A was used, the explants were left on medium A for 2–4 days and then were transferred to medium B or differentiation medium. If medium B was used, the explants were cultured for 3 weeks and then transferred to differentiation medium. The differentiation medium was the same as that used for immature embryo culture.

Chromosome counting

Root tips of test-tube plantlets were used as material for chromosome counting. Before fixation, the material was pretreated in cold water (1 °C–3 °C) for 24 h and then in 0.02%–0.05% colchicine solution at room temperature for 4 h. After hydrolysis, material was stained with Feulgen reagent and squashed in a drop of acetocarmine.

Results

Necrosis Test I

The F_1 progeny of the following crosses (all *T. aestivum* × *T. durum*) were grown in the greenhouse for necrosis test:

CMB-2 × CMD-1
CMB-2 × CMD-7
CMB-2 × CMD-8
CMB-6 × CMD-1
CMB-6 × CMD-7
CMB-6 × CMD-8

All the crosses with CMB-2 were necrotic while all the crosses with CMB-6 were normal. Reciprocal crosses of all the above combinations produced the same results. The necrotic syndrome was severe, i.e. when seedlings reached the two to three-leaf stage, the necrotic syndrome was obvious and at any period beyond this stage, only the youngest leaf and part of the second youngest leaf were green (but much smaller than normal plants), while older leaves turned brown and died. About 1 month after germination, the necrotic plants were very weak and they never flowered. These results indicate that CMB-2 is a Ne^s carrier and CMB-6 is a non-carrier, while all three *T. durum* lines are Ne^s carriers.

Recovery (Re) mutants

Young embryos of CMB-2 × CMD-8 were cultured and 153 plantlets were regenerated. When these plantlets were grown in the greenhouse, most of them expressed the necrotic syndrome just like the F_1 seedlings. However, among 67 successfully transferred regenerates, two mutants were found. They were named Re1 (Fig. 1) and Re2 mutants (Re: recovery). Re1 and Re2 grew normally and no necrotic syndrome could be detected. They tillered more than normal varieties but the leaves were not as large as normal lines. Re1 and Re2 grew normal spikes and experiments showed they were male sterile and could not set self-pollinated seeds.

Later the above experiment was repeated in a slightly modified way. Young embryos of CMD-7 × CMB-2 were cultured and, among 47 regenerates, a new Re mutant (Re3) was found. All three Re mutants are morphologically identical.

In order to maintain Re mutants, young ears of an Re1 plant were cultured and regenerates were obtained and grown to maturity. In this way Re1 has been propagated for four asexual "generations".

Cytological observation has demonstrated that Re1 has only 34 chromosomes (Fig. 2) instead of the 35 chromosomes expected for F_1 progeny of *T. aestivum* × *T. durum*.

When pollinated with normal lines of *T. aestivum* or *T. durum*, Re1 did set seeds which can germinate and grow to plants. This means that the aneuploidy only causes male sterility, not female sterility.

Necrosis test II

Using Re1 as female, we backcrossed it with its parental lines CMB-2 and CMD-8/CMD-7 (CMD-7 is not the parental line of Re1, but is very similar to CMD-8). Here we use BC1 to represent "backcross generation 1". Table 1 shows the results of necrosis test of the BC1 plants.

Table 1 shows that when Re1 is backcrossed with the *T. aestivum* parental line, all the progeny are normal. On the other hand, in about half of the crosses with T. durum, Ne carriers are necrotic and the other half are normal.

All normal Re1 × CMB-8 BC1 plants are partially fertile and set some seeds that can germinate and grow

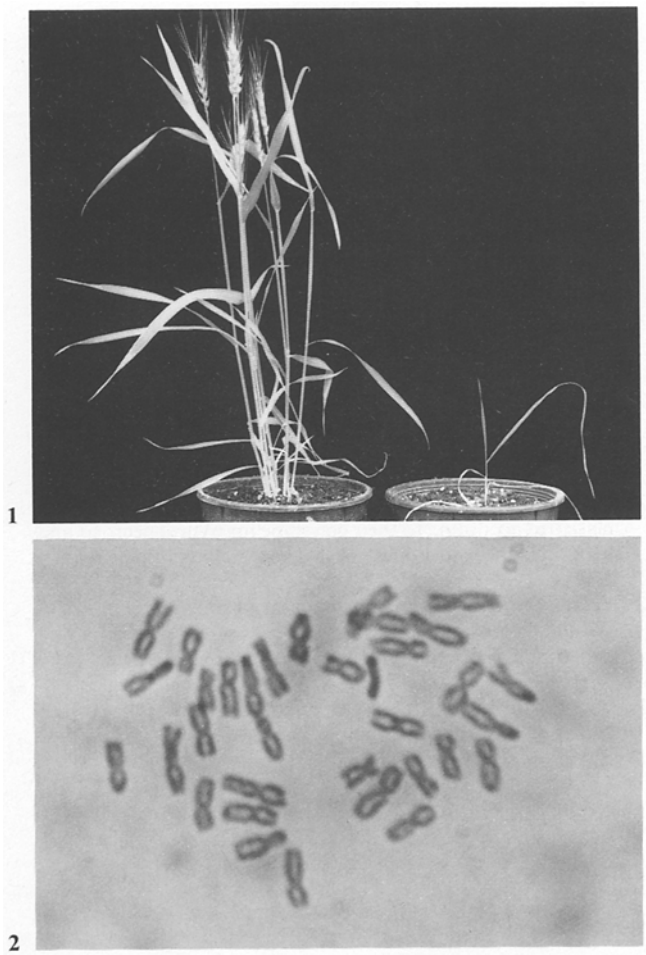

Fig. 1. *Right*: a necrotic plant (F1 of *T. aestivum* × *T. durum*). *Left*: a flowering plant of Recovery mutant Re1

Fig. 2. Chromosomes of a root tip cell from Recovery mutant Re1 ($2n = 34$)

Table 1. Necrosis test of some BC1 plants from Re1

Crosses	Total germinated BC1 seed	Necrotic plants	Normal plants
Re1 × CMB-2	9	0	9
Re1 × CMB-8	7	3	4
Re1 × CMB-7	8	5	3

normally. The extent of fertility varies with different individuals. The other two BC1 lines are still growing.

Conclusion and discussion

Nature of Re mutants

Because Re1 has only 34 chromosomes instead of the expected 35 chromosomes, the natural assumption is that during the culture process, one of the two chromosomes (2B or 5B) which carries Ne genes was lost.

Using Ne(D) to represent the Ne gene from *T. durum* and Ne(B) for Ne gene from *T. aestivum*, we can expect the following two outcomes: (1) The lost chromosome is from *T. durum*. When Re1 is backcrossed to CMB-2 [Ne(B) carrier], we have Ne(B) × Ne(B)Ne(B), i.e. the BC1 generation should be all normal since no complementary necrosis gene Ne(D) is present in this combination. When Re1 is backcrossed to CMD-7 or CMB-8 [Ne(D) carrier], we have Ne(B) × Ne(D)Ne(D), in which case half of the BC1 plants should be necrotic and the other half should be normal, because after meiosis only half of the egg cells of Re1 contain Ne(B) gene. (2) The lost chromosome is from *T. aestivum*. Using a similar rationale as in above, when Re1 is backcrossed to CMB-2, half of the progeny should be necrotic and half normal; when Re1 is backcrossed to CMD-7 or CMD-8, all the progeny should be normal.

Based on our results (Table 1), the lost chromosome in Re1 is from *T. durum*, i.e. CMD-8. As it is known that Ne1 is extremely common in durum wheat and Ne1 is located on chromosome 5B, Re1-regenerated plants probably lost chromosome 5B of CMD-8 during the immature embryo culture process.

The use of Re mutants

Re mutants appear in about 1% of the regenerates from the immature embryo culture of the necrotic crosses. This is an acceptable percentage since the regeneration from culture is quite easy. In addition, the Re mutants can be maintained and propagated through young ear culture. When we combine these culture methods, the necrosis problem can be bypassed. BC1 plants derived from the backcross of Re1 with CMD-8 set a number of seeds (BC2 seeds). BC2 plants grow normally and most of them are morphologically similar to CMD-8. By combining selection with traditional breeding methods and/or anther culture of the BC1 or BC2 generation, we can definitely use the necrotic cross CMB-2 × CMD-8 in a breeding program.

Chromosome number variation in tissue culture

Chromosome number variation in tissue culture has been documented for a long time although the mechanism is not clear. In this study, because the necrotic syndrome can be easily detected, it becomes a selectable marker for chromosome elimination. Re mutants can be picked out without difficulty leading to their use in a breeding program. During the process of culturing the immature embryos of the necrotic combination between CMB-2 and CMD-8/CMD-7, chromosomes that don't carry Ne genes might be lost in the same way, but we cannot detect

these changes easily. If each chromosome has an equal chance of being lost, the overall frequency of one particular chromosome missing during culture should be about 17%.

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