the region holds sufficient diversity to distinguish the sequences of the groups in the above two cases.

Thus, the application of computational tools in sequence analysis can be useful in extracting patterns of different types. This study demonstrates one such utility by considering the most consistent repeating pattern. The pattern search, in this case, has even yielded supplementary information that is vital to distinguish the groups from each other. Likewise, using different logic for pattern search and with 16S rRNA as a model, the generated knowledge would provide better understanding of the molecular mechanisms of ribosome function as well as the relationship of organisms on an evolutionary scale.

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ACKNOWLEDGEMENTS. This work was supported by a grant from the Department of Biotechnology, New Delhi and CSIR Network Program (SM0002). We thank Mr G. Balamurgan, Mr M. Ravichandran, Mr A. Padmanabhan, Mr M. Parthiban and Ms S. Sugunadevi, Bharathiar University, Coimbatore, who developed the group-wise database on 16S rRNA as part of their project study.

Received 28 August 2003; revised accepted 10 March 2004

Decline in reactive oxygen species at low light intensity can overcome necrosis barrier in hybrid wheat

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In an attempt to overcome the barrier to genetic transfer in wheat (*Triticum aestivum*), necrotic hybrids Kalyansona × C306, WL711 × C306, J24 × C306) showing different degrees of necrosis were grown in phytotron along with their parents Kalyansona, WL711, J24 and C306 under lower light intensity (25% of that in the field), long days and higher mean temperatures compared to those existing in the field. Leaf hydrogen peroxide levels, pollen viability and characters from ears of hybrids and their parents were determined. Leaves from plants grown in phytotron revealed lower levels of hydrogen peroxide in comparison to those in the field. Hybrids and their parents grown in phytotron exhibited early flag leaf and ear emergence than those seen in the field. Less severely necrotic hybrids formed viable seeds in the developing ears on the plant itself, whereas the most severely necrotic hybrid of the three formed seeds only after culturing in a medium. Main shoot ear from hybrids was smaller than that of the parents and revealed lower spikelet number, grain number, total grain weight and weight per grain when compared to parents. Thus, lowering of reactive oxygen species content at low light intensity coupled with long days and higher mean temperatures enabled the hybrids to complete their life cycle that resulted in overcoming the necrosis barrier in F_1 progeny in wheat.

HYBRID necrosis is a phenomenon wherein premature and gradual death of leaves and leaf sheaths occurs in certain wheat hybrids[1]. It results due to two complementary genes, *Ne1* and *Ne2*. Multiple allelism of these two genes leads to various degrees of necrosis in different crosses. Transfer of desirable traits into well-adapted genetic backgrounds is therefore dependent on the genetic constitution of the donor and recepient parents at the *Ne* loci. A natural mutant selection of wheat, C306 is recognized as the most widely adapted source for drought tolerance in wheat, which does not form viable hybrids with most high yielding wheat lines due to its genetic constitution[2], *Ne1Ne1ne2ne2*. Drought-resistant *Ne1* carrier wheat cultivar C306 (C) was crossed with high yielding *Ne2* carrier cultivars, Kalyansona (K), WL711 (W) and J24 (J). These exhibited different degrees of hybrid necrosis. Under field conditions, hybrids of Kalyansona × C306 (K × C)

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reached up to the 6–8 leaf stage, WL711 × C306 (W × C) survived up to flag leaf emergence stage, while J24 × C306 (J × C) died after ear emergence. Our earlier studies had established ear culture as a technique to overcome the expression of necrosis in crosses that survived up to flag leaf[3], which suggested that potentially the necrotic complementation could be overcome in wheat by facilitating vegetative growth by manipulating the environment where the hybrid grew. Since plants from the hybrid K × C died even before reaching the flag leaf stage, ear culture was not feasible for obtaining seeds from this cross.

Previous studies from our laboratory have shown that hybrid necrosis is a genetically programmed cell death process involving oxidative stress. The leaves of F_1 hybrids grown in the field showed higher levels of superoxide ion[4] and lipid peroxidation[5], even before the onset of visible necrosis. Light intensity, mean temperatures and photoperiod encountered by wheat plants in the field during vegetative growth are about 1200–1500 μmol $m^{-2}s^{-1}$, 16°C D/10°C N and 10–12 h respectively. An increase in the mean temperature and photoperiod leads to shortening of the vegetative phase and hastens ear emergence[6], while lowering of light intensity reduces the generation of reactive oxygen species in plants[7]. Thus, the three hybrids along with their parents were grown under controlled environmental conditions in the phytotron at low light intensity, higher mean temperatures and long days compared to the field, to study the effect of change in the environment on the life cycle of the hybrid plants.

The seeds of the three hybrids K × C, W × C and J × C along with their parents were sown in pots containing sand, peat and vermiculite in the ratio 1 : 2 : 1. There were twenty pots for each genotype, while for K × C, double the number of pots was maintained. Two plants were maintained per pot. The pots were kept in growth chambers (Controlled Environments Limited, Canada) at the National Phytotron Facility, Indian Agricultural Research Institute, New Delhi. Chambers were maintained at a light intensity of 340 μmol $m^{-2}s^{-1}$ (about 25% of that of the field). Growth conditions such as relative humidity (70%), photoperiod (11 h/day), mean temperatures (20°C D/15°C N) were uniformly maintained in the chamber. Full strength Hoagland nutrient solution was added every alternate day. At 45 days after sowing, the mean temperatures and photoperiod in phytotron chamber were raised to 23°C D/19°C N and 13 h respectively. The three hybrids and their parents were also grown in the field as described earlier[8].

H_2O_2 content was determined in the fifth leaf of all the hybrids and their parents by the method of Warm and Laties[9], with slight modification. Sampling of hybrid leaf was done at two stages – at full expansion, before onset of necrosis (stage I) and when 50% leaves were necrotic (stage II). Leaves of parents at corresponding stages were also taken. Frozen leaf material (200 mg) was ground in 1 ml of ice cold 5% TCA. The crude extracts were centrifuged at 12,000 *g* for 30 min. An aliquot of the supernatant was passed through a column of Dowex anion exchange resin to remove the coloured components from the extract. The amount of H_2O_2 in the resulting extract was tested by pipetting 50 μl test solution into 50 μl of 0.5 mM luminol (3-aminopthalhydrazide) in 0.2 M NH_4OH (pH 9.5) in 1 ml cuvettes. The cuvettes were placed in the measuring cell of the luminometer (Thermo Labsystems, Finland) and chemiluminescence was initiated by adding 100 μl of 0.5 mM $K_3Fe(CN)_6$ (in 0.2 M NH_4OH). The emitted photons were integrated at 3 s intervals for 40 s. H_2O_2 content was calculated from a standard curve.

Viability of the pollen from the main shoot ear was examined by the staining sensitivity reaction of pollen grains with iodine[10]. Anthers from ears of hybrids and their parents were collected before pollen dehiscence and were slit open. Staining of pollen grains was completed within 5 min of the collection of ears. Pollen grains that stained dark brown were classified as viable and those that remained transparent were sterile.

After ear-emergence, the ears from K × C plants were cultured in a medium containing sucrose (5%), glutamine (0.04%) and antioxidants, i.e. reduced ascorbate (0.5 mM) and reduced glutathione (0.1 mM), as previously reported[3].

The ears from plants grown in phytotron were taken to maturity and ear length, spikelet number, grain number, total grain weight and weight/grain were recorded for hybrids and their respective parents. Five plants constituted a replicate. There were three replicates for each measurement. The data were analysed statistically and standard error was calculated. The values of hybrids were compared with the parental mean, i.e. average of both the parents was used.

Hybrid plants grown in both the environments (i.e. field and phytotron) exhibited progressive necrosis in their leaves while the leaves of parents were normal. Comparison of H_2O_2 levels in the leaves from hybrids and their parents revealed lower H_2O_2 levels in the plants grown at lower light intensity and higher temperatures in phytotron compared to those of the field. However, H_2O_2 levels in the hybrids were higher even before the appearance of visible necrosis when compared to parents and further increased with the progression of necrosis both in the field and phytotron (Table 1). Before visible necrosis, H_2O_2 content in the leaves of hybrids K × C, W × C and J × C was about 65.8, 62.8 and 69.9% respectively, of those of the field-grown hybrids. When the hybrid leaves were about 50% necrotic, H_2O_2 levels in phytotron-grown hybrids K × C, W × C and J × C were about 58.1, 42.2 and 70.9% respectively, of those of the field-grown hybrids. Lower levels of H_2O_2 in phytotron could be attributed to low light intensity compared to the field, as low light intensity decreases the production of reactive oxygen species[7,11].

Table 1. H_2O_2 levels (nmol g FW^{-1}) in the fifth leaf of necrotic hybrids (Kalyansona × C306–K × C, WL711 × C306–W × C and J24 × C306–J × C) and their parents (Kalyansona–K, WL711–W, J24–J and C306–C) from field (light intensity, 1200–1500 μmol m^{-2} s^{-1}; mean temperatures, 16°C D/10°C N; photoperiod, 10–12 h) and phytotron (light intensity, 340 μmol m^{-2} s^{-1}; mean temperatures, 20°C D/15°C N; photoperiod, 11 h, relative humidity, 70%)

	K	K × C	W	W × C	J	J × C	C
Before visible necrosis							
Field	468 ± 78	1365 ± 235	524 ± 74	1087 ± 196	550 ± 75	776 ± 76	484 ± 84
Phytotron	402 ± 61	899 ± 105	398 ± 61	683 ± 179	428 ± 92	543 ± 122	407 ± 78
At 50% necrosis							
Field	603 ± 95	4178 ± 587	621 ± 48	3284 ± 567	558 ± 73	1814 ± 105	588 ± 69
Phytotron	512 ± 76	2426 ± 432	496 ± 81	1386 ± 368	456 ± 87	1287 ± 118	506 ± 76

Values are mean of three replicates ± SE.

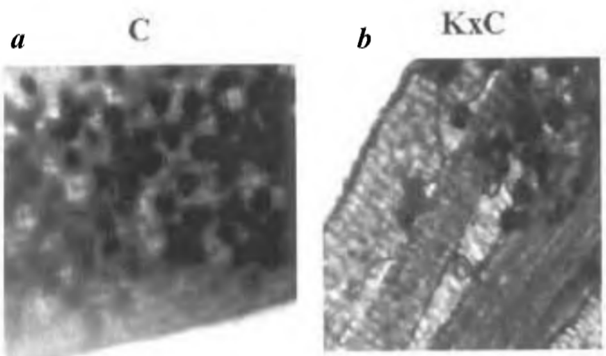

Figure 1. Anthers from the ears of Kalyansona × C306 (K × C) (***b***) and its parent C306 (C) (***a***) grown at light intensity 340 μmol m^{-2} s^{-1}, showing pollen grains stained with iodine. Magnification 8.3 × 10 × photomicrograph on Olympus SZ60 STR microscope. Relative humidity, mean temperatures and photoperiod were maintained at 70%, 23°C D /19°C N and 13 h respectively.

Flag leaf emergence was seen in parents and hybrids between 55 and 65 days after sowing in the phytotron compared to 70 and 80 days after sowing in the field. In phytotron all the plants of parents and hybrids W × C and J × C reached flag leaf emergence, whereas only 43% plants of K × C reached flag leaf emergence. Similarly, plants grown in phytotron exhibited early ear emergence (75–80 days after sowing) compared to those in the field (90–95 days after sowing). All plants of parents reached ear emergence in phytotron. However about 15, 55 and 85% respectively, of hybrids K × C, W × C and J × C reached ear emergence. Early flag leaf emergence could have resulted due to a combination of higher mean temperatures (23°C D/19°C N) and long photoperiods (13 h) during the vegetative phase of development in plants grown in phytotron compared to the field[6,12]. All ears except that of K × C developed normally on plants and produced seeds. At the ear emergence stage, the F_1 plants of the K × C cross were almost dead, but the ears could be retrieved and cultured. However, only 66% of these cultured ears produced seeds.

Pollen viability studies from the ears of hybrids and their parents grown in phytotron at 340 μmol $m^{-2}s^{-1}$ revealed that pollen grains from parents and hybrids W × C and J × C were alive (represented by C in Figure 1 *a*), as they stained dark brown[10]. However, ears from K × C showed presence of few, viable pollen (Figure 1 *b*). A possible reason for this low viable pollen count could be the presence of higher levels of reactive oxygen species and lipid peroxidation (unpublished results), as male sterile anthers in rice have also been shown to exhibit higher H_2O_2 and lipid peroxidation than fertile anthers[13]. The higher reactive oxygen species levels in the developing ear of K × C may also have influenced the seed-setting process.

Ear length and spikelet number of the main shoot ear for all the hybrids were less than those of their respective parents in phytotron (Table 2). The main shoot ear of all the hybrids exhibited lower grain number, total grain weight and individual grain weight compared to those of the parents (Table 2). Grain number from the main shoot ear in hybrids K × C, W × C and J × C was about 26, 42.8 and 48.8% respectively, of those of the parental mean. The three hybrids exhibited only about 15.6, 37.1 and 42.2% respectively of the total grain weight of the parental mean. Hybrids W × C and J × C maintained about 87.2 and 85.8% respectively of the weight per grain compared to the parents. Thus, we report here the production of seeds from the ears of necrotic wheat hybrids W × C and J × C directly on the plant without any ear culture step.

The seeds produced by the hybrid K × C could be divided into three categories depending on the weight of the individual grain. Category I included seeds varying in individual grain weight between 13 and 18 mg. Grain weight of category II seeds varied between 9 and 13 mg. Category III included shrivelled and disfigured seeds weighing less than 9 mg (Figure 2). Mean for all the three categories was taken to represent the weight/grain, which was about 60.5% of that of the parental mean (Table 2). Lower grain number and total grain weight and weight/grain from the main shoot ear in hybrids could be attri-

Table 2. Ear characters from the main shoot ear of necrotic hybrids (K × C, W × C and J × C) and their parents (K, W, J and C) grown in phytotron (light intensity; 340 μmol m^{-2} s^{-1}, mean temperatures, 23°C D/19°C N; photoperiod, 13 h; relative humidity, 70%)

	K	K × C	W	W × C	J	J × C	C
Ear length (cm)	23.4 (± 4.2)	6.5 (± 2.4)	24.6 (± 5.1)	8.4 (± 2.8)	22.5 (± 4.6)	8.8 (8.8 3.1)	23.8 (± 5.4)
Spikelet number/ear	13.6 (± 3.6)	7.6 (± 2.5)	13.4 (± 2.8)	10.2 (± 2.1)	14.8 (± 2.9)	9.8 (± 2.4)	15.3 (± 3.2)
Grain number/ear	20.4 (± 2.2)	5.6 (± 2.6)*	22.1 (± 3.2)	9.6 (± 2.0)	21.6 (± 3.9)	10.8 (± 1.8)	22.6 (± 4.1)
Total grain weight (mg)/ear	351 (± 81)	57.3 (± 21)*	362 (± 48)	138 (± 21)	336 (± 56)	151 (± 35)	381 (± 52)
Weight/grain (mg)	17.2 (± 3.5)	10.3 (± 5.6)*	16.3 (± 2.6)	14.4 (± 2.6)	15.5 (± 3.6)	13.9 (± 2.7)	16.8 (± 3.5)

Values represent mean ± SE (n = 3). Values are mean of three replicates ± SE.
*Mean of three categories of seeds obtained in K × C cross.

Figure 2. Seeds obtained from ears of hybrid Kalyansona × C306 (K × C) and its parents Kalyansona (K) and C306 (C) grown at 340 μmol m^{-2} s^{-1} light intensity, 70% relative humidity, 23°C D/19°C N mean temperatures and 13 h photoperiod. Seeds of three different categories (I, II and III) differing in individual grain weights were obtained from the ears of K × C.

buted to the poor nutritional status in hybrids as the topmost photosynthetically active leaves were undergoing necrosis. Germination studies revealed 100% germination of category I seeds, while only 33% seeds of category II showed germination and none of the seeds of category III germinated. F_2 plants segregated into necrotic and non-necrotic plants. The non-necrotic F_2 plants exhibited recombination of traits such as plant height and flag leaf area, which differed considerably in the parents. We have previously shown that the cross W × C segregated in the ratio 9 : 7 in F_2 for necrosis : healthy plants.

Thus, hastening the development by coupling higher temperatures with long days and lowering oxidative stress in phytotron by lowering the light intensity, coupled with ear culture, we report here the production of seeds from a severely necrotic wheat cross K × C. Production of seeds from the hybrids of necrotic wheat crosses W × C and J × C directly from the plant was also achieved without any ear culture step.

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ACKNOWLEDGEMENT. This work was supported by funds obtained from National Agricultural Technology Project, Indian Council of Agricultural Research, New Delhi.

Received 13 November 2003; revised accepted 23 April 2004