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Differential response of antioxidant enzymes in leaves of necrotic wheat hybrids and their parents

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The leaves of necrotic hybrid of wheat (*Triticum aestivum* L.) exhibited high superoxide content associated with increased lipid peroxidation and membrane damage in earlier studies (Khanna-Chopra et al. 1998, Biochem Biophys Res Commun 248: 712–715; Dalal and Khanna-Chopra 1999, Biochem Biophys Res Commun 262: 109–112). In the present study, we investigated the activities of the antioxidant enzymes in the leaves of necrotic wheat hybrids, Kalyansona × C306 (K × C) and WL711 × C306 (WL × C) and their parents at different developmental stages. The K × C hybrid exhibited more severe necrosis than WL × C. In K × C, superoxide dismutase (SOD) activity showed no increase over the parents, while WL × C showed an early increase, but it was possibly insufficient to scavenge increased superoxide. Activities of guaiacol peroxidase, ascorbate peroxidase and glutathione reductase were enhanced, while catalase exhibited a decrease in activity, with the appearance of visible necrosis in both the hybrids. The isozyme profile of the antioxidant enzymes was similar in the hybrids and their parents. One existing isoform of guaiacol peroxidase showed an early appearance in the hybrid and increased in intensity with the progression of necrosis. The results reveal a differential response of antioxidant enzymes in necrotic wheat hybrids as compared to their parents. The response differed in magnitude at developmental stages of the leaves, which might be related to the intensity of necrosis expressed by the hybrids.

Introduction

Combining drought tolerance with high yield potential to sustain yield under water limiting conditions is an important objective in agriculture. However, there are certain wheat crosses that are desirable and yet unsuccessful. Wheat (*Triticum aestivum*) var. C306, known to be drought tolerant (Sinha et al. 1986), was crossed with high yielding var. Kalyansona and WL711, but the crosses were unsuccessful. The F1 from both crosses showed a severe degree of necrosis, and plants died at different stages of development without completing their life cycle. This phenomenon is called hybrid necrosis.

Hybrid necrosis is the premature gradual death of leaves and leaf sheath in certain wheat hybrids. It is caused by two complementary genes, *Ne1* and *Ne2*, when brought together in hybrid combination (Hermsen 1963). The intensity of necrosis varies greatly due to multiple allelism of these necrotic genes, which are localized on the long arm of 5B and short arm of 2B chromosomes, respectively (Nishikawa et al. 1974). Many promising wheat varieties are carriers of *Ne1* or *Ne2*, which limits the parental choice for transfer of desirable traits (Zeven 1981, Bijral et al. 1990). A detailed analysis of the mechanism of hybrid necrosis is required to understand the mechanism of cell death in plants, as well as to overcome this barrier in gene transfer in wheat.

We reported that necrotic leaves of wheat hybrid showed an increased level of superoxide anion, which preceded the onset of visible necrosis, and the gradient of superoxide generation in the leaf was parallel to the progression of necrosis (Khanna-Chopra et al. 1998). Increased superoxide concentration was also associated with higher lipid peroxidation and membrane permeability (Dalal and Khanna-Chopra 1999), which are the common symptoms of oxidative stress.

In the last few years, considerable evidence has accumulated implicating active oxygen species (AOS) and their scavenging system in various cell death mechanisms. The

Abbreviations – AOS, active oxygen species; APX, ascorbate peroxidase; CAT, catalase; GR, glutathione reductase; GSSG, oxidized glutathione; POD, peroxidase; PVP-40, polyvinylpyrrolidone-40; SOD, superoxide dismutase.

activities of the superoxide and H_2O_2 producing enzymes are enhanced, while activity of H_2O_2 scavenging enzymes is depressed during senescence and hypersensitive response (Levine et al. 1994, Pastori and del Rio 1997). Since different stresses may influence the cellular organelles or cells differently, organisms have evolved multiple molecular forms of various antioxidant enzymes that are located in different organelles (Tsang Ed et al. 1991). Studies on transgenic plants indicate that the ability of plants to metabolize AOS depends on the co-ordination of the overall protective system operating in the cell, rather than on a single enzyme activity, for preventing damage induced by oxidative stress (Sen Gupta et al. 1993, Slooten et al. 1995).

In the present study, we analyzed the enzymes involved in the metabolism of AOS at different stages of leaf development in both the wheat hybrids and their parents. As the intensity of necrosis expressed by the two wheat hybrids was different, a comparative study was carried out in order to investigate whether there is any correlation between intensity of necrosis with the induction of different antioxidant enzymes. We also studied the changes in the isozyme profile of these enzymes, in order to ascertain the induction of new isoform and/or changes in the activity of already existing isoforms in necrosis.

Materials and methods

Plant material and growth conditions

Wheat (*Triticum aestivum* L.) cv. C306 was crossed individually with cvs Kalyansona and WL711. The F1 seeds of both crosses, i.e., Kalyansona × C306 (K × C) and WL711 × C306 (WL × C), along with their parents, were sown in the field of the Water Technology Center, Indian Agriculture Research Institute, New Delhi, India, on 15th November 1997. The plant-to-plant and row-to-row distances were 10 cm and 25 cm, respectively. Fertilizer was given at the rate of 100:50:50 NPK kg ha^{-1} as a single basal dose. The plants were allowed to grow under natural field conditions and irrigated to avoid development of water deficit. All the parameters were measured in the fourth leaf from the base of the plant from hybrids and their parents. The leaf samples were collected on 4, 8, 10, 12, 14 and 16 days of leaf emergence.

Enzyme assays

Leaf tissue was ground to fine powder in liquid nitrogen and then homogenized in 1.5 ml of 50 m*M* sodium phosphate buffer (pH 7.0) containing 2 m*M* EDTA, 5 m*M* β-mercaptoethanol and 4% (w/v) Polyvinylpyrrolidone-40 (PVP-40; Donahue et al. 1997). The homogenate was centrifuged at 30000 *g* for 30 min at 4°C. The supernatant was dispensed in aliquots and was stored at −80°C until further analysis. Protein extraction for determining ascorbate peroxidase (APX, EC 1.11.1.11) was performed as described above, except that the homogenization buffer also contained 5 m*M* ascorbate. Protein content was determined according to Lowry et al. (1951), using bovine serum albumin as standard.

Total superoxide dismutase (SOD, EC 1.15.1.1) activity was measured spectrophotometrically based on inhibition in the photochemical reduction of nitroblue tetrazolium (Beauchamp and Fridovich 1971). The 1-ml reaction mixture contained 50 m*M* phosphate buffer (pH 7.8), 58 μM nitroblue tetrazolium, 2.4 μM riboflavin and 9.9 m*M* methionine and 0.025% (v/v) Triton X-100. One unit of SOD is defined as the amount of enzyme that inhibited the nitroblue tetrazolium (NBT) reduction by 50%. APX (EC 1.11.1.11) was determined by following the decrease in ascorbate at A_{290} as described by Nakano and Asada (1981). Glutathione reductase (GR, EC 1.6.4.2) was estimated by monitoring the oxidation of NADPH at 340 nm according to Schaedle and Bassham (1977). Corrections were made by subtracting values obtained in the absence of either substrate or enzymatic extracts. Peroxidase (POD, EC 1.11.1.7) activity was determined at 470 nm for 3 min in a reaction mixture containing 50 m*M* sodium phosphate buffer (pH 6.9), 3.2 m*M* guaiacol and 0.4 m*M* H_2O_2 (Chance and Maehly 1955). Catalase (CAT, EC 1.11.1.6) activity was measured by following the consumption of H_2O_2 at 240 nm. The 3-ml reaction mixture contained 50 m*M* sodium phosphate buffer (pH 7.0) and 10 m*M* H_2O_2 (Aebi 1984).

Native polyacrylamide gel electrophoresis (PAGE) and activity staining

Protein from both the hybrids and their respective parents at all the stages were subjected to discontinuous PAGE under non-denaturing and non-reducing conditions. Electrophoretic separation was performed at 4°C using Mini protean electrophoresis unit (Bio-Rad Laboratories, Hercules, CA, USA). An equal amount of protein was loaded on the gel. SOD isoforms were visualized by incubating the gel in 50 m*M* phosphate buffer containing 0.24 m*M* NBT and 28 μM riboflavin for 20 min in the dark and then immersing the gel in 28 m*M* TEMED followed by exposure to a light source at room temperature (Beauchamp and Fridovich 1971). SOD from horseradish (Sigma-Aldrich, USA; S4636) was used as standard. Staining for POD isoforms was achieved by incubating the gel in sodium acetate buffer (200 m*M*, pH 5.0) containing 2 m*M* Benzedine. The reaction was initiated by adding 3 m*M* H_2O_2 (Seevers et al. 1971). GR isoforms were detected by incubating the gels in Tris-HCl (pH 7.5) containing 0.24 m*M* monotetrazolium, 0.34 m*M* 2,6-dichlorophenolindophenol, 3.6 m*M* oxidized glutathione (GSSG) and 0.4 m*M* NADPH. Duplicate gels were assayed for GR activity, one with and one without GSSG (Anderson et al. 1990). GR, type VI from spinach (Sigma-Aldrich; G3011), was used as standard. APX was detected in gel according to Mittler and Zilinskas (1993). The carrier buffer contained 2 m*M* ascorbate, and the gels were pre-run for 30 min to allow ascorbate to enter the gel prior to the application of samples. After the separation, the gels were incubated in 50 m*M* sodium phosphate buffer (pH 7.0) containing 2 m*M* ascorbate for 30 min. This was followed by another 20-min incubation in 50 m*M* sodium phosphate buffer (pH 7.0) containing 4 m*M* ascorbate and 2 m*M* H_2O_2. The gels were

washed with buffer for 1 min. Isoforms were visualized by submerging the gel in a solution of sodium phosphate buffer (pH 7.8) containing 28 m*M* TEMED and 2.45 m*M* NBT. Activity staining for CAT was performed following the method described by Woodbury et al. (1971). CAT from bovine liver (Sigma-Aldrich; C9322) was used as standard. Gels were rinsed in double distilled water followed by incubation in 0.003% (v/v) H_2O_2 for 10 min. After a brief rinse, the CAT isozymes were detected by incubating the gel in 1% ferric chloride and 1% potassium ferricyanide solution.

Statistics

The results are expressed as means with standard error (SE). The significant difference between mean values of antioxidant enzyme activities of individual hybrid and its parents was evaluated by randomized complete block design, two factor factorial analysis of variance. Differences were considered to be significant at $P \leq 0.01$. Asterisks (*) indicate significant difference in enzyme activities between cvs × developmental stages in a cross.

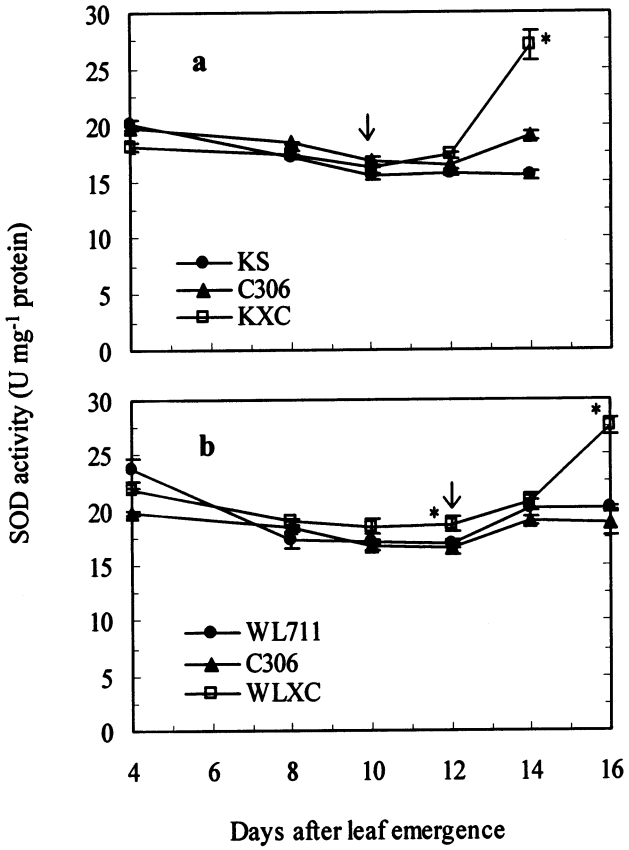

Fig. 1. Changes in total SOD activity in the leaves of necrotic wheat hybrids K × C (a), WL × C (b) and their parents during leaf development. SOD activity was measured from the fourth day of leaf emergence until the hybrid leaf was almost dead. The arrow indicates the onset of necrosis in the hybrid leaf. Values are mean ± SE of 3 replicates. The asterisk (*) represents significant differences between the hybrid and its parents across developmental stages.

Results

The degree of necrosis in the two hybrids was severe, since both died before ear emergence. However, the hybrids differed in their intensity of necrosis. This was evident both at the plant and leaf level. K × C survived only up to the 6–8-leaf stage, while WL × C survived up to the flag leaf stage. Similarly, necrosis appeared earlier, i.e., before full expansion, in K × C leaf as compared to WL × C, where it started after full expansion of leaf. On the visual scale, K × C hybrid leaves were 25% necrotic on day 10, which progressed to 50 and 75% necrosis on day 12 and day 14 of leaf emergence, respectively. The leaves of WL × C hybrid showed 25% necrosis on day 12, progressing to 50 and 75% necrosis on day 14 and 16, respectively.

Superoxide dismutase (SOD)

No significant difference in SOD activity was observed between K × C and its parents, at the beginning of leaf emergence and even after the onset of necrosis. The activity increased significantly ($P \leq 0.01$) in the last developmental stage, when the hybrid leaf was almost 75% necrotic (Fig. 1a). In WL × C hybrid, SOD activity was marginally higher than the parents before the onset of necrosis. The activity increased significantly from 12 ($P \leq 0.05$) to 41% ($P \leq 0.01$) over that of the parents after 12 days and 16 days of leaf emergence, which represented the beginning and advanced stages of necrosis in WL × C leaves (Fig. 1b).

Ascorbate peroxidase (APX)

There was a significant increase ($P \leq 0.01$) in the APX activity in K × C leaves as compared to the parents after 8 days of leaf emergence (Fig. 2a). After the onset of necrosis, APX activity increased from 29% to a maximum of 69% ($P \leq 0.01$) over the parents after 14 days of leaf emergence. In WL × C leaves, APX activity was similar to that of the parents up to 12 days after leaf emergence. Significant increase ($P \leq 0.01$) was observed only at 14 and 16 days after leaf emergence, where APX activity showed an increase of 16 and 78% over that of its parents (Fig. 2b).

Glutathione reductase (GR)

GR activity in both hybrids did not show any significant change as compared to the parents before the appearance of necrosis. After the onset of necrosis, GR activity in K × C leaf was significantly ($P \leq 0.01$) higher, i.e., 57 and 85% compared to the parents, after 12 and 14 days of leaf emergence, respectively (Fig. 3a). WL × C leaf showed a significant increase ($P \leq 0.01$) in GR activity during leaf development, coinciding with the appearance of necrosis after 12 days of leaf emergence (Fig. 3b). The necrotic leaf of WL × C exhibited 3-fold ($P \leq 0.01$) higher GR activity as compared to the parents after 16 days of leaf emergence (Fig. 3b).

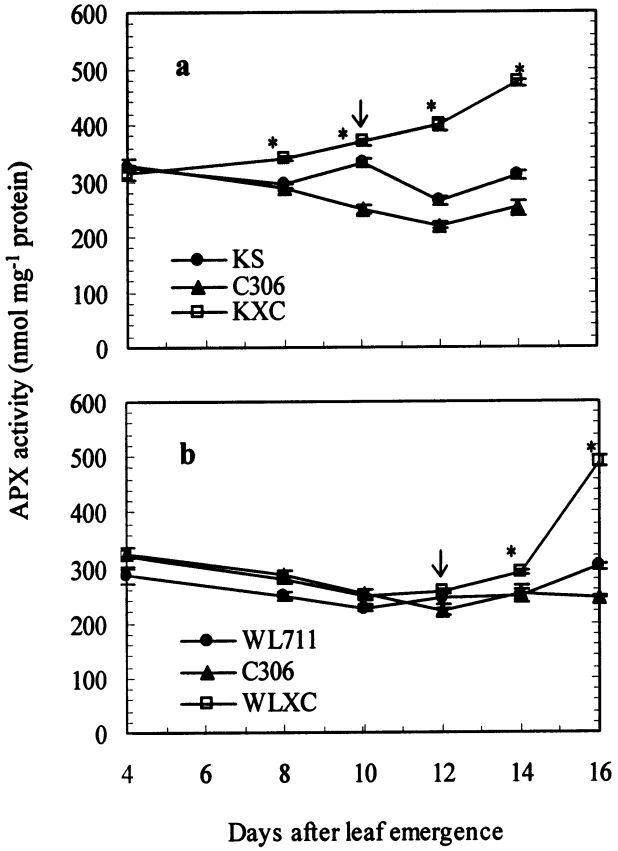

Fig. 2. Changes in APX activity in the leaves of necrotic wheat hybrids K × C (a), WL × C (b) and their parents during leaf development. APX activity was measured from the fourth day of leaf emergence until the hybrid leaf was almost dead. The arrow indicates the onset of necrosis in the hybrid leaf. Values are mean ± SE of 3 replicates. The asterisk (*) represents significant differences between the hybrid and its parents across developmental stages.

Peroxidase (POD)

K × C hybrid showed a significantly higher ($P \leq 0.01$) POD activity as compared to the parents from the beginning of leaf emergence (Fig. 4a). This increase was 4–8- and 10.5-fold at 10, 12 and 14 days after leaf emergence. In WL × C, the appearance of necrosis was associated with increased POD activity. POD activity of WL × C was 1.9- and 5-fold ($P \leq 0.01$) higher at 12 and 16 days after leaf emergence as compared to its parents (Fig. 4b).

Catalase (CAT)

CAT activity increased in the parents from the beginning of leaf emergence to leaf maturation (Fig. 5a,b). The onset of necrosis was accompanied by a decline in CAT activity in both the hybrids. CAT activity in K × C reduced to 69% as compared to its parents after 14 days of leaf emergence (Fig. 5a), while in WL × C, it was reduced to 87% as compared to its parents after 16 days of leaf emergence (Fig. 5b).

Isozyme profile of antioxidant enzymes

Superoxide dismutase

On electrophoretic separation, 4 major bands were observed (Fig. 6). Gels pre-treated in 2 m*M* KCN and 5 m*M* H_2O_2 followed by staining for SOD activity indicated isoform-I to be Mn-SOD and the other 3 were identified as CuZn-SOD (Fig. 7). The isozyme pattern was similar in both the hybrids and their parents. In K × C, no major changes were observed in the intensities of SOD isoforms up to 10 days after leaf emergence. Intensities of all the isoforms increased after 12 days of leaf emergence (Fig. 6b). In WL × C, all the isoforms showed enhancement in their intensities after the appearance of necrosis, i.e., 12 days after leaf emergence (Fig. 6a).

Ascorbate peroxidase

Activity staining for APX showed two isoforms (Fig. 8). APX-II contributed more to the total APX activity in both hybrids and their parents. In K × C, the increase in APX activity was evident from 8 days after leaf emergence, since both isoforms showed an increase in intensity as compared to the parents (Fig. 8b). In WL × C, the intensity of the isoforms was enhanced only at 14 and 16 days of leaf

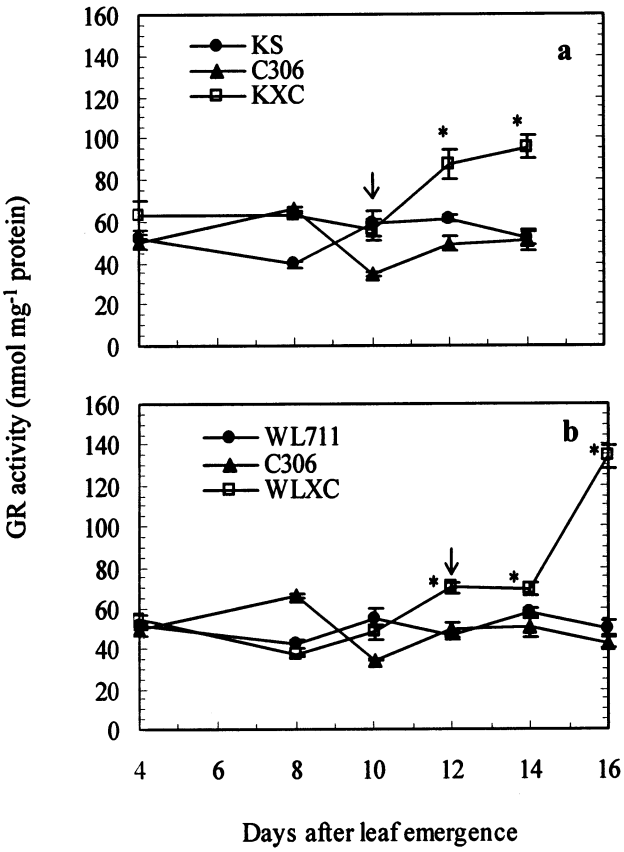

Fig. 3. Changes in GR activity in the leaves of necrotic wheat hybrids K × C (a), WL × C (b) and their parents during leaf development. GR activity was measured from the fourth day of leaf emergence until the hybrid leaf was almost dead. The arrow indicates the onset of necrosis in the hybrid leaf. Values are mean ± SE of 3 replicates. The asterisk (*) represents significant differences between the hybrid and its parents across developmental stages.

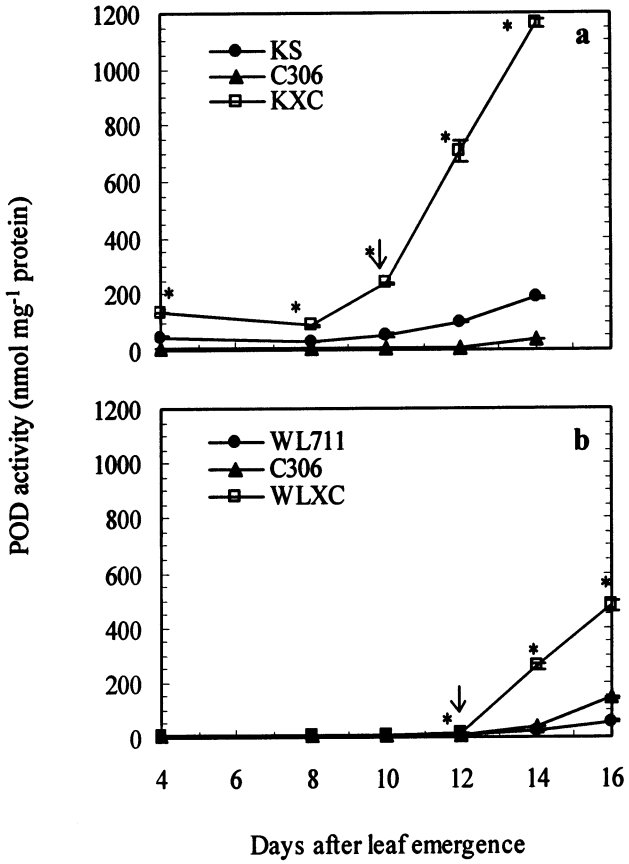

Fig. 4. Changes in guaiacol POD activity in the leaves of necrotic wheat hybrids K × C (a), WL × C (b) and their parents during leaf development. Guaiacol POD activity was measured from the fourth day of leaf emergence until the hybrid leaf was almost dead. The arrow indicates the onset of necrosis in the hybrid leaf. Values are mean ± SE of 3 replicates. The asterisk (*) represents significant differences between hybrid and its parents across developmental stages.

emergence (Fig. 8a). With the progress of necrosis, APX-I was preferentially increased in both hybrids.

Glutathione reductase

The isozyme profile of both the hybrids and their parents was similar (Fig. 9). Five isoforms were observed; two isoforms were GSSG-specific, while 3 were GSSG-non-specific. The activity of the GR-II was more than GR-I at all stages in the hybrids and their parents. The activity of all the existing isoforms increased after the onset of necrosis in both the hybrids (data is shown for WL × C hybrid and its parents only).

Peroxidase

The isozyme pattern of POD exhibited 5 bands (Fig. 10). The isoform POD-II was observed in the hybrids at the beginning of leaf emergence and increased linearly with necrosis. C306, a common parent in both the hybrids, exhibited the POD-II isoform at 10 days after leaf emergence, while it was either marginal or absent in other parents at all the developmental stages examined. The intensity of POD IV and POD V was higher at all the stages in the hybrids. In the parents, the intensity of POD IV was negligible, and POD V showed an increase with leaf development (Fig. 10).

Catalase

Activity staining for CAT showed only one isoform. The intensity of the isoform decreased with onset of necrosis in both K × C (Fig. 11) and WL × C (data not shown).

Discussion

The increased level of superoxide anion, higher lipid peroxidation and membrane damage in the hybrid leaves (Khanna-Chopra et al. 1998, Dalal and Khanna-Chopra 1999) indicated an oxidative environment in cells undergoing necrosis. Increased accumulation of AOS in elicited cell cultures has been shown to enhance membrane damage and cell death (Rusterucci et al. 1996). The accumulation of superoxide anion preceding necrotic lesion formation during hypersensitive response and induction of tumor formation by wild type *Agrobacterium tumefaciens* has been observed (Jabs et al. 1996, Jia et al. 1996). An increase in SOD

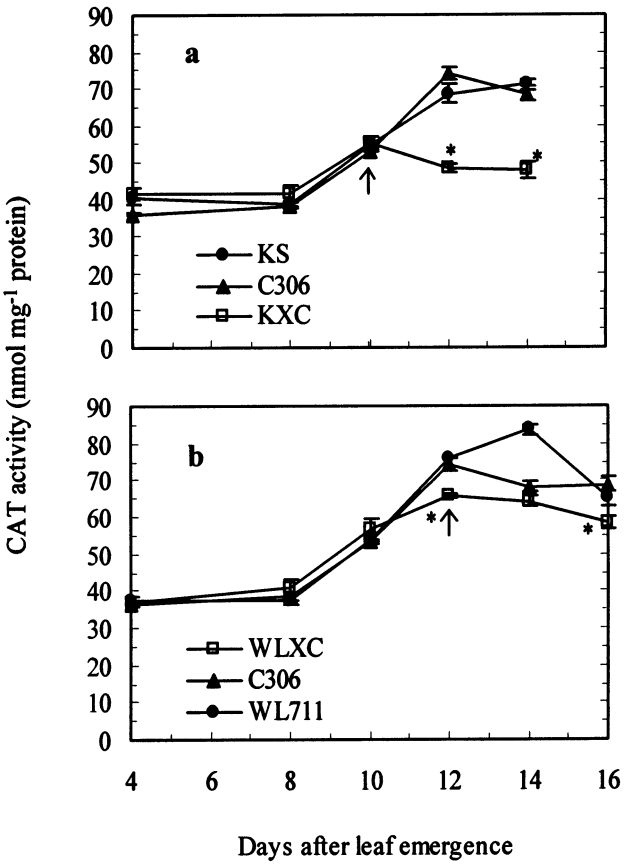

Fig. 5. Changes in CAT activity in the leaves of necrotic wheat hybrids K × C (a), WL × C (b) and their parents during leaf development. CAT activity was measured from the fourth day of leaf emergence until the hybrid leaf was almost dead. The arrow indicates the onset of necrosis in the hybrid leaf. Values are mean ± SE of 3 replicates. The asterisk (*) represents significant differences between hybrid and its parents across developmental stages.

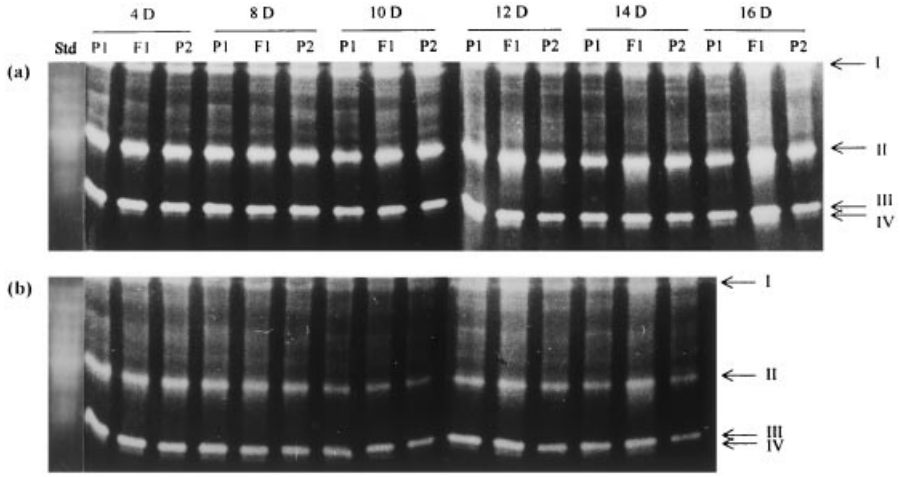

Fig. 6. Native gels stained for the activity of SOD in the leaves of necrotic wheat hybrids WL × C (a) and K × C (b) with their parents during the leaf development. (a) P1, WL711; F1, W × C; P2, C306. (b) P1, KS; F1, K × C; P2, C306. (D represents days after leaf emergence.) The arrows indicate the isoforms. Equal amounts of proteins (40 μg) were loaded in each lane of the gel. Std refers to standard SOD from horseradish (Sigma-Aldrich).

activity reduced the lesion formation and delayed tumor development. Transgenic plants overexpressing SOD also showed increased resistance to oxidative stress (Sen Gupta et al. 1993, McKersie et al. 1999). Moreover, SOD gene expression seems to be modulated by the rate of oxyradical formation (Bowler et al. 1992). In the present study, in spite of increased superoxide level, the SOD activity in K × C hybrid did not show any significant change over its parents, except at the last stage (Fig. 1). In contrast, WL × C leaves exhibited a rise in SOD activity at full expansion, when superoxide content was 2.8-fold higher than the parents (Khanna-Chopra et al. 1998). The increase in SOD activity was not sufficient to prevent necrosis, although it could possibly delay the onset of necrosis. Hence, non-responsiveness of SOD in necrotic hybrids could be responsible for the appearance and development of necrosis.

The next step in enzymatic antioxidant defense involves H_2O_2 degrading enzymes, such as POD, CAT and ascorbate peroxidase-glutathione reductase (APX-GR). Our results show that these enzymes respond differently to the change in cellular environment. Thus APX and POD showed an increasing trend, while CAT activity declined after onset of necrosis in the hybrid leaf (Figs. 2, 4 and 5). The differences were also expressed in terms of the time and magnitude of induction of the enzymes. In K × C hybrid, APX and POD increased before the appearance of necrotic lesions, while in WL × C, increase in both the enzymes was observed only after the onset of necrosis (Figs. 2 and 4). In some studies, increased activity of antioxidant enzymes was observed with the onset of visible injury (Pitcher et al. 1992, Van Camp et al. 1994). GR, which acts in conjunction with APX to metabolize H_2O_2 through the Halliwell-Asada pathway, followed a similar trend to that of APX in WL × C, while in K × C, GR activity increased only at the advanced stage of necrosis (Fig. 3).

The antioxidant enzymes are inductive in nature (Foyer et al. 1994). Recently, H_2O_2 has been shown to induce cytosolic APX (Morita et al. 1999). Therefore, an increase in the activity of APX and POD suggested increased production of H_2O_2 in the cell. CAT plays a significant role in scavenging H_2O_2, as transgenic plants with reduced CAT activity showed development of necrotic lesions under high light intensity (Chamnongpol et al. 1996, Takahashi et al. 1997, Willekens et al. 1997). In the present study, decrease in CAT activity and PSII efficiency also showed a correlative occurrence at visible onset of necrosis (Fig. 5, Dalal and Khanna-Chopra 1999). Studies on transgenic CAT-1 deficient tobacco plants revealed that CAT-1 was essential for protection of the ascorbate and glutathione pool from oxidation for maintaining the redox balance in cells (Willekens et al. 1997). Thus, availability of ascorbate and reduced glutathione may become a rate limiting step during antioxidant defense (Foyer et al. 1994, 1995).

In the present study, an early increase of POD activity and its isoform POD II, which shows preferential increase with necrosis, suggests the possibility of POD as a source of H_2O_2. PODs can generate H_2O_2 by oxidation of NADH, NADPH, thiols and certain phenols (Halliwell 1978, Mader and Amberg-Fisher 1982, Pichorner et al. 1992) and have been shown to be involved in elicitor induced H_2O_2 production and lipid peroxidation (Vera-Estrella et al. 1992, Bolwell et al. 1995). Therefore, it is worth investigating whether POD-II has any relation with necrosis or is just an attribute of hybridization. The role of PODs is complicated by their involvement in diverse physiological functions. An increase

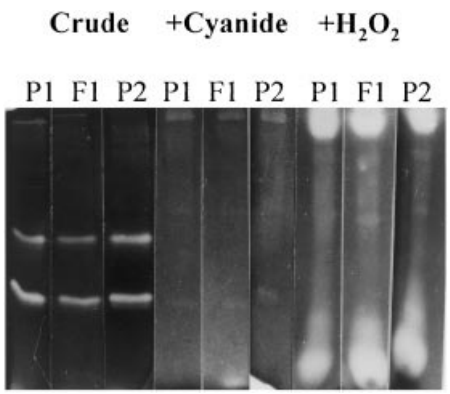

Fig. 7. Native gels stained for identifying different isoforms of SOD. Gels pre-treated in 2 m*M* KCN and 5 m*M* H_2O_2 followed by staining for SOD activity indicated isoform-I to be Mn-SOD and the other 3 were identified as CuZn-SOD. P1, WL711; F1, WL × C306; P2, C306.

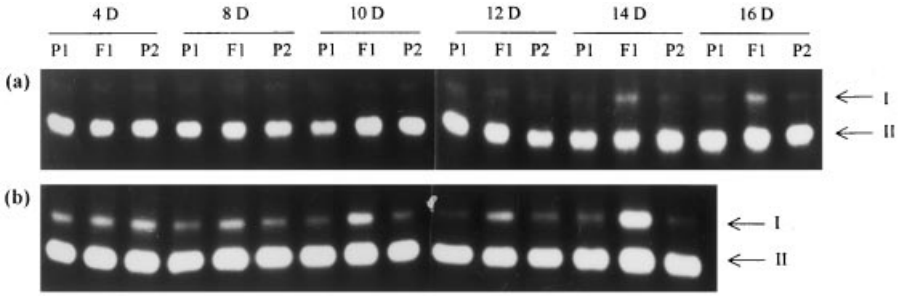

Fig. 8. Native gels stained for the activity of APX in the leaves of necrotic wheat hybrids WL × C (a) and K × C (b) with their parents during the leaf development. (a) P1, WL711; F1, WXC; P2, C306. (b) P1, KS; F1, K × C, P2, C306. (D represents days after leaf emergence.) The arrows indicate the isoforms. Equal amounts of proteins (40 μg) were loaded in each lane of the gel.

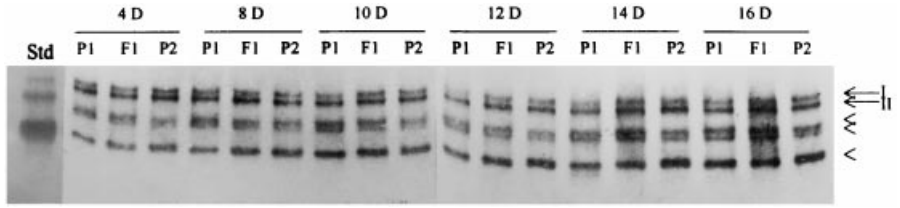

Fig. 9. Native gels stained for the activity of GR in the leaves of necrotic wheat hybrid WL × C with its parents during the leaf development. P1, WL711; F1, WL × C, P2; C306. (D represents days after leaf emergence.) Arrows indicate the GSSG specific isoforms. Arrowheads indicate the GSSG non-specific isoforms. Equal amounts of proteins (40 μg) were loaded in each lane of the gel. Std refers to standard GR type VI from spinach (Sigma-Aldrich).

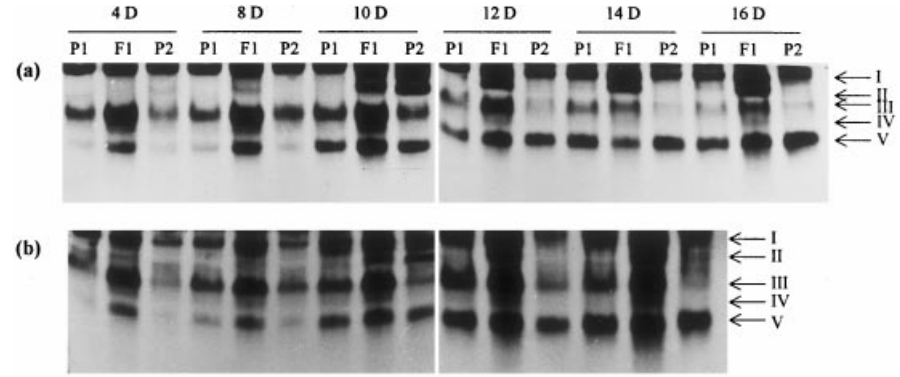

Fig. 10. Native gels stained for the activity of POD in the leaves of necrotic wheat hybrids WL × C (a) and K × C (b) with their parents during the leaf development. (a) P1, WL711; F1, WXC; P2, C306. (b) P1, KS; F1, K × C; P2, C306. (D represents days after leaf emergence.) The arrows indicate the isoforms. Equal amounts of proteins (40 μg) were loaded in each lane of the gel.

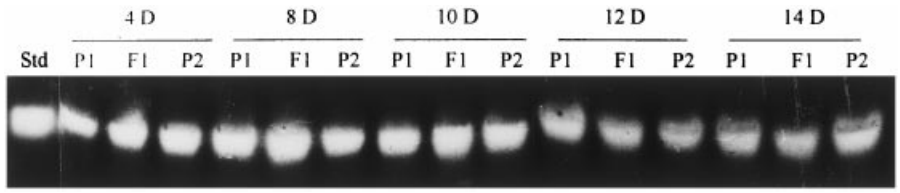

Fig. 11. Native gels stained for the activity of CAT in the leaves of necrotic wheat hybrid, K × C with its parents during the leaf development. P1, KS; F1, K × C; P2, C306. (D represents days after leaf emergence.) Equal amounts of proteins (10 μg) were loaded in each lane of the gel. Std refers to standard CAT from bovine liver (Sigma-Aldrich).

in PODs has been reported in various stresses and has been linked with protection from oxidative damage, lignification and cross linking of cell wall, so as to prevent spread of infection during pathogen attack (Brisson et al. 1994). Yet the reason for increased POD during senescence or aging is not known and merits further study (Panavas and Rubinstein 1998).

In conclusion, hybrid necrosis in wheat leaves is associated with oxidative stress not accompanied by a well co-ordinated antioxidant defense system. There was a preferential increase in the activities of PODs, while SOD activity showed a limited increase as compared to the parents during the progression of necrosis. The decline in CAT activity could be an important factor in establishment of necrosis in hybrid leaves. The differential response of the antioxidant enzymes in the wheat hybrids with respect to the stage of leaf development may be contributing to the differences in the intensity of necrosis exhibited by the two hybrids. Currently, experiments are being conducted to study the interplay of various components of active oxygen metabolism at subcellular level and also to understand the molecular-genetic basis of hybrid necrosis in wheat.

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