SHORT COMMUNICATION

Leaf senescence in rice plants: cloning and characterization of senescence up-regulated genes

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Abstract

To identify senescence-associated genes (SAGs) in rice leaves, senescence was induced by transferring rice seedlings into darkness. Senescence up-regulated cDNAs were obtained by PCR-based subtractive hybridization. Among 14 SAG clones characterized, 11 were found to be associated with both dark-induced and natural leaf senescence. Three clones were associated only with dark-induced leaf senescence. The possible physiological roles of these SAGs during rice leaf senescence are discussed.

Key words: Dark-induced senescence, gene expression, natural leaf senescence, rice.

Introduction

Leaf senescence represents an endogenously controlled degenerative process that ultimately leads to organ death. It progresses in an age-dependent manner, but is also affected by a complex interaction of developmental age with other internal and external factors (Noodén, 1988a; Smart, 1994). The external factors include temperature, drought, nutrient deficiency, shading, wounding, and pathogen infection. The internal factors include developmental stages and phytohormone levels (Hensel et al., 1993). Leaf senescence is regulated by the co-ordinated expression of specific genes, and many senescence-associated genes (SAGs) have been identified (Buchanan-Wollaston, 1997). The encoded gene products are principally involved in degradation or remobilization of biomolecules, but are also involved in protecting cell viability for completion of the senescence process. Many SAGs have functions that remain unknown. Some are possibly involved in triggering senescence or controlling the progression rate of senescence. Analysing the various modes of SAG regulation should provide insights into the molecular mechanisms of the leaf senescence programme.

Although leaf SAGs have been studied in a number of plant species such as Arabidopsis thaliana (Lohman et al., 1994), barley (Kleber-Janke and Krupinska, 1997), maize (Smart et al., 1995), Brassica napus (Buchanan-Wollaston and Ainsworth, 1997), and tomato (John et al., 1997), reports on leaf senescence in rice are limited mainly to physiological and cytological studies (Lutts et al., 1996; Inada et al., 1998, 1999). Leaf senescence in rice starts from the lower leaves and extends upward as the plant grows. The loss of assimilatory capacity as leaf senescence progresses contributes to limited grain yield (Noodén, 1988b), and delayed leaf senescence may increase crop productivity. This paper reports the isolation of SAG clones from the senescing leaves of dark-grown rice plants. The identities, patterns of gene expression during dark-induced and natural leaf senescence, and the possible physiological significance of these SAG clones are discussed.

Materials and methods

Seeds of Japonica rice (Oryza sativa L. cv. Tainong 67) were imbibed in water for 2 d and planted in moist compost mix in a growth chamber equipped with cool-white fluorescent lamps. The photoperiod was 14/10 h light/dark at 30 °C with 85% humidity. After 8 d, the seedlings were transferred to complete darkness to induce leaf senescence. The green control was grown under the same photoperiod. The second leaf blade was harvested at different time points after dark treatment. For natural leaf senescence studies, the rice plants were grown in a greenhouse under natural light conditions. The leaf blades were harvested at different stages of development and senescence (Y, G, S1, S2, S3, S4).

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Senescence-associated cDNA fragments were generated by subtractive hybridization using the PCR-Select cDNA subtraction kit (Clontech, USA). Poly (A)$^+$ RNA was purified from pooled RNA of senescing leaves of rice seedlings after 1, 1.5, 2, and 3 d of dark incubation using an oligo (dT)-cellulose column (Pharmacia Biotech, Sweden). Poly (A)$^+$ RNA was also prepared from the green control. Two populations of cDNA were synthesized, hybridized, and subjected to PCR amplification. The differentially expressed PCR products were cloned into pGEM-T Easy vector (Promega Corp, USA). To eliminate the false positives, reverse Northern analysis was performed. The duplicate slot-blot membranes carrying inserts of cDNA clones were hybridized, one to a total cDNA probe from green leaves and the other to a total cDNA probe from dark-induced senescing leaves. The cDNA clones that showed enhanced expression during leaf senescence were sequenced by an ABI PRISM 377 DNA sequencer (USA). Homology search against sequence databases was performed by using the BLAST program at the National Center for Biotechnology Information, USA. To study the expression pattern of each SAG clone, total RNA was extracted from leaves and different organs as described previously (Chen et al., 1995) and Northern analysis was performed according to To et al. (To et al., 1999). The membrane was hybridized with a $^{32}$P-labelled cDNA insert and specific gene probe for 258 rRNA, and visualized with PhosphorImager (Molecular Dynamics, USA).

Results and discussion

Fourteen senescence-associated cDNA clones which have been confirmed by reverse Northern analysis were selected for characterization (Table 1). These up-regulated cDNAs were identified to be mainly associated with amino acid metabolism (OsI2, Osh36, OsI20, OsI55, OsI30), fatty acid metabolism (OsI57, OsI85), protein degradation (OsI295), and stress response (OsI43, Osh70). Most of these SAG clones have not been previously reported as leaf-senescence associated genes. Several clones (Osh69, OsI139, Osh67) have functions that remain unknown.

Figures 1 and 2 show the gene expression profiles of these SAGs during dark-induced leaf senescence and during natural leaf senescence. Among these clones, 11 were found to be associated with natural leaf senescence in a developmental age-dependent manner. Three clones (Osh36, Osh43, Osh70) encoding aminotransferase, salt-induced protein, and polygalacturonase, respectively, were associated only with dark-induced senescence but not with natural leaf senescence. This observation is consistent with other findings (Park et al., 1998) that certain SAGs are differentially regulated when leaf senescence is induced by different senescence-inducing factors. Furthermore, the levels of transcripts represented by these SAG clones are quite different in the two leaf-senescence systems. For example, transcripts represented by OsI295 are of high abundance during dark-induced senescence and of rather low abundance during natural senescence. Conversely, Osh69 transcripts accumulate to much higher levels during natural senescence. The expression of these SAGs was also examined in different organs (Fig. 3). The induced expression of OsI85 and Osh67 transcripts was found to be highly specific in naturally senescent leaves, but not in other organs tested.

The expression levels of OsI2, OsI20, and OsI55 that encode enzymes aminotransferase, branched-chain $\alpha$-keto dehydrogenase, and $\beta$-methylcrotonyl-CoA carboxylase for amino acid metabolism were found to be significantly enhanced at the stages of natural leaf senescence (S1–S3). Relatively late induction of OsI30 transcripts

<table>
<thead>
<tr>
<th>Clone</th>
<th>GenBank accession no.</th>
<th>cDNA (bp)</th>
<th>mRNA (kb)</th>
<th>Sequence homology to$^a$</th>
<th>Sequence identity$^b$ (%)</th>
<th>Up-regulated during natural senescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>OsI2</td>
<td>AF251073</td>
<td>799</td>
<td>2.0</td>
<td>Aminotransferase (Capsicum chinense), AF085149</td>
<td>69 (231)</td>
<td>+</td>
</tr>
<tr>
<td>Osh36</td>
<td>AF251070</td>
<td>567</td>
<td>2.0</td>
<td>Alanine-glyoxylate aminotransferase homolog (Arabidopsis thaliana), AF166352</td>
<td>80 (188)</td>
<td>–</td>
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<td>OsI20</td>
<td>AF251067</td>
<td>258</td>
<td>1.6</td>
<td>El-$\alpha$ subunit of branched-chain $\alpha$-keto dehydrogenase (tomato), Z94180</td>
<td>75 (85)</td>
<td>+</td>
</tr>
<tr>
<td>OsI55</td>
<td>AF251074</td>
<td>656</td>
<td>2.6</td>
<td>Biotinylated subunit of $\beta$-methylcrotonyl-CoA carboxylase (soybean), U08469</td>
<td>55 (203)</td>
<td>+</td>
</tr>
<tr>
<td>OsI30</td>
<td>AF251065</td>
<td>264</td>
<td>1.6</td>
<td>4-Hydroxyphenylpyruvate dioxygenase (barley), AJ000693</td>
<td>89 (38)</td>
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<td>OsI57</td>
<td>AF251076</td>
<td>533</td>
<td>1.8</td>
<td>3-Ketoacyl-CoA thiolase (mango), X75329</td>
<td>81 (177)</td>
<td>+</td>
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<tr>
<td>OsI85</td>
<td>AF251075</td>
<td>491</td>
<td>2.1</td>
<td>Isocitrate lyase (Brassica napus), Y13356</td>
<td>83 (74)</td>
<td>+</td>
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<td>OsI295</td>
<td>AF251164</td>
<td>562</td>
<td>2.0</td>
<td>Aspartic protease (rice), D32164</td>
<td>90 (119)</td>
<td>+</td>
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<td>OsI381</td>
<td>AF251077</td>
<td>773</td>
<td>1.7</td>
<td>dTDP-glucose 4,6-dehydratase homolog (Arabidopsis thaliana), Z49239</td>
<td>91 (231)</td>
<td>+</td>
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<td>Osh69</td>
<td>AF251068</td>
<td>315</td>
<td>2.7</td>
<td>Seed imbibition protein (Brassica oleracea), X79330</td>
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<td>AF251071</td>
<td>864</td>
<td>2.2</td>
<td>Seed protein B32E (barley), X64254</td>
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<tr>
<td>Osh67</td>
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<td>3.0</td>
<td>Unknown</td>
<td>+</td>
<td></td>
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<td>OsI43</td>
<td>AF285163</td>
<td>545</td>
<td>0.7</td>
<td>Salt-induced protein (rice), S45168</td>
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<tr>
<td>OsI70</td>
<td>AF251069</td>
<td>523</td>
<td>2.0</td>
<td>$\beta$-subunit of polygalacturonase isoenzyme 1 (tomato), U63374</td>
<td>65 (112)</td>
<td>–</td>
</tr>
</tbody>
</table>

$^a$The most similar protein is according to the NCBI Gapped BLAST and PSI-BLAST search results.

$^b$The number of the amino acid residues shown in parenthesis are the amino acids deduced from each clone used for sequence comparison.
encoding 4-hydroxphenylpyruvate dioxygenase (HPPD) for degrading aromatic amino acids was observed at the senescent stage S3. HPPD expression has been reported to be induced during barley leaf senescence (Kleber-Janke and Krupinska, 1997). Apparently amino acid degradation and nitrogen remobilization are very important biochemical events which occur during leaf senescence.

Two clones (Osl2, Osl85) that are involved in fatty acid degradation and remobilization are those encoding 3-ketoacyl CoA thiolase and isocitrate lyase. The expression levels of both corresponding transcripts were remarkably enhanced at the same senescent stage S3 and declined again at the S4 stage, suggesting that these two genes may be co-ordinately regulated by the senescence-inducing factors during rice leaf senescence.

Osl295 showed highest identity (90%) with an aspartic proteinase, oryzasin 1, from Japonica rice cv. Nipponbare. Evidence has indicated that aspartic proteinase belongs to a multiple gene family in rice plants (Asakura et al., 1995), and Osl295 may represent a new member in the gene family. A number of SAGs coding for proteinases have been identified as being associated with leaf senescence. These include cysteine proteinase of Arabidopsis (Lohman et al., 1994), serine proteinase of parsley (Jiang et al., 1999), and aspartic proteinase of Brassica (Buchanan-Wollaston and Ainsworth, 1997).

Fig. 1. Northern blot analyses of SAG mRNAs that increase in abundance during dark-induced leaf senescence of rice seedlings. G refers to green leaves of the rice seedlings grown under a 14:10 h light:dark photoperiod. 1D, 1.5D, 2D, 3D, and 4D refer to leaves of rice plants after 1 d, 1.5 d, 2 d, 3 d, and 4 D of dark incubation, respectively. RNA (30 µg) was loaded in each lane of a 1% agarose gel. The blot was hybridized with a different 32P-labelled cDNA insert of SAG clone as designated on the left. The autoradiograms were analysed with a PhosphorImager with exposure times approximately the same for all blots. Rice 25S rRNA gene probe was used to verify the equal loading, and the corresponding exposure time was reduced about 100-fold.

Fig. 2. Northern blot analyses of SAG mRNA that increases in abundance during natural leaf senescence of the greenhouse-grown rice plants. Northern blots carrying RNA (30 µg) isolated from leaves at different stages of development and senescence were hybridized with 32P-labelled DNA inserts from the different SAG clones designated on the left. Y refers to young leaf rolls of a 1-month-old plant; G refers to fully expanded green leaves (100% Chl) of a 2-month-old plant; S1 refers to senescing leaves (80–95% Chl) of the plant at the stage of panicle development; S2 refers to senescing leaves (60–80% Chl) of the plant at the flowering stage; S3 refers to senescing leaves (45–60% Chl) of the plant at the stage of panicle development; and S4 refers to senescing leaves (30–45% Chl) of the plant at the seed maturity stage. The autoradiograms were analysed with a PhosphorImager. Exposure times varied for different SAGs in order to get suitable band intensities and were indicated as a number of folds (1 x, 2 x, 4 x) for comparison.
Elucidation of the intracellular location, kinetics of accumulation, and substrate specificities of these proteins will delineate their roles during the senescence process.

OsI381 showed high homology to a putative dTDP-glucose 4,6-dehydratase from A. thaliana. The expression of OsI381 transcripts was found to be up-regulated at the early senescent stage (S1). dTDP-glucose 4,6-dehydratase was originally identified in Salmonella (Jiang et al., 1991), which is involved in the biosynthesis of the cell wall polysaccharides. Although several plant cDNAs homologous to dTDP-glucose 4,6-dehydratase have been cloned, the biological functions of the corresponding gene products in plants are still unknown.

Two up-regulated cDNA clones (Osh69, OsI139) showed high homology to a seed imbibition protein from B. oleracea and seed protein B32E from barley, respectively. Another cDNA clone Osh67 that exhibited specific expression in senescing leaves (Fig. 3) shared no significant homology with any known gene. Functional identification is needed to get an insight into the physiological roles that these genes play during leaf senescence.

The clones involved in stress response are those encoding salt-induced protein (OsI43) and polygalacturonase (Osh70). OsI43 showed 98% identity with salT encoding a 15 kDa salt-induced protein of Indica rice cv. Taichung Native 1. SalT gene was reported to be expressed in rice roots and sheaths in response to salt stress and drought (Claes et al., 1990), but the biochemical function has not been identified. Osh70 showed 65% identity with the β-subunit of polygalacturonase isoenzyme 1 from tomato. The transcripts of both OsI43 and Osh70 were induced in the leaves of rice seedlings after dark incubation, but no detectable mRNA signal for OsI43 or Osh70 was observed in the naturally senescing leaves. The quick induction of Osh70 mRNA expression during dark-induced leaf senescence (Fig. 1) implies that polygalacturonase may play a role in the dark-induced senescing tissue by generating oligogalacturonides as signals to activate some defence-response genes (Côté and Hahn, 1994). It has been reported that a number of SAGs that are defence-related genes were induced during leaf senescence (Quirino et al., 1999). These observations suggest an overlap in the molecular events of plant defence responses and the leaf senescence programme.

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References


