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Salicylic Acid and Hydrogen Peroxide in Abiotic Stress Signaling in Plants

By

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S u m m a r y

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During the 1990's, much evidence has suggested roles for salicylic acid and H_2O_2 in responses of plants to pathogens. This article reviews recent international research suggesting that these compounds are also involved in responses to abiotic stresses such as extreme temperatures, ozone pollution or UV irradiation. We recently discovered that salicylate applied at low concentrations (1-100 μM) could protect seedlings or cultured microplants against heat-shock. Endogenous levels of salicylic acid and its glucoside have been shown to increase in plants subjected to heat, ozone or UV. Salicylic acid and H_2O_2 appear to influence each other's levels in plant tissues. Increases in H_2O_2 occur in plants subjected to many abiotic stresses, but it has not been established to what extent this H_2O_2 is a symptom or a signal of abiotic stress. Consistent with a signaling role for this compound, some reports have shown that treatment with H_2O_2 can protect plants against cold- or heat-stress, and can induce stress-response genes.

I n t r o d u c t i o n

Plants frequently suffer stressful interactions with their environment. These include biotic stresses such as pathogens and pests, and abiotic stresses such as temperature extremes, non-optimal water or light conditions, or chemical pollution. During the 1990's, important discoveries have been made about the biochemical mechanisms by which plant cells signal to their neighbours that they are suffering stress. Early in the decade, evidence emerged that salicylic acid (SA),

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considered only a minor plant growth regulator for many years, was involved in the induction of disease resistance. Subsequently, increasing evidence has indicated that the signal-response to pathogen attack involves a system generating hydrogen peroxide (H_2O_2), with which SA interacts (KAUSS & JEBLICK 1996, MUR & al. 1997). This article reviews recent evidence suggesting that these two compounds, SA and H_2O_2, may also be involved in signaling responses to abiotic stresses.

Salicylic Acid and Abiotic Stress

The discovery that salicylate could induce thermotolerance was made by chance during potato tissue culture research. Inclusion of the artificial SA analogue acetylsalicylic acid (ASA) in the culture medium of microplants of potato (*Solanum tuberosum* L.) causes potentially useful effects such as tuberization. Heat treatments are used in protocols for elimination of viruses from potato microplants, and it was noticed that microplants cultured on ASA were more tolerant of such heat treatments (LOPEZ-DELGADO & al. 1998). The protective ASA concentrations (1-10 µM) were very low and typical of true hormonal responses. Further experiments compared SA-treatments with heat-acclimation in compost-grown plants. DAT & al. 1998b showed that spraying with 10-100 µM SA induced a similar transient thermotolerance (1-4 h post-treatment) to a moderate, acclimatory heat-treatment in 8-day-old seedlings of mustard (*Sinapis alba* L.). Moreover, DAT & al. 1998a found that heat-acclimation increases endogenous levels of SA and its glucoside, e.g. levels were 4-fold higher after 30 min at the acclimation temperature. Endogenous levels of SA (15-120 µM) were comparable to the concentrations effective as thermoprotective spray applications. This is different to the induction of systemic disease resistance by SA, for which unphysiological concentrations of 0.5-2 mM are needed.

Heat is not the only abiotic stress in which SA has been implicated. Plants carrying an SA-hydroxylase transgene (to deplete endogenous SA) exhibited increased sensitivity to ozone (SHARMA & al. 1996). Increases in endogenous SA were observed in plants treated with ozone or UV light (YALPANI & al. 1994, SHARMA & al. 1996), and in catalase-deficient transgenic plants under high light (CHAMNONGPOL & al. 1998). This could explain why SA-inducible pathogenesis-related (PR) genes also appeared during these abiotic stresses (YALPANI & al. 1994, SHARMA & al. 1996, CHAMNONGPOL & al. 1998). Heat can also induce certain PR genes (MARGIS-PINHEIRO & al. 1994).

The best characterized SA-inducible genes are PR genes, but there is evidence for others consistent with a role for SA in a wider range of stress responses. Salicylic acid may affect the expression of genes for glutathione S-transferases, which are involved in protection against oxidative stress (CHEN & al. 1996, SHARMA & al. 1996), and it enhances the activity of the alternative pathway of respiration, which may prevent harmful production of free radicals in mitochondria (WAGNER 1995). GOLDSBROUGH & al. 1993 found SA-inducible

binding of a tobacco nuclear protein to a 10 bp sequence motif conserved amongst PR genes and a range of abiotic-stress genes.

A common factor linking pathogenesis with abiotic stresses is suggested by experiments of STROBEL & KUC 1995, who found that the herbicide paraquat, which causes oxidative damage, could induce systemic disease resistance, while conversely pathogen inoculation or SA treatment could protect against paraquat damage. The possible relationships between SA and oxidative damage, disease and abiotic stress responses will be discussed in the next section.

Hydrogen Peroxide and Abiotic Stress

Studies on how SA might interact with stress signaling mechanisms have identified a complex relationship with H_2O_2. Salicylate can increase H_2O_2 levels in plant tissues (RAO & al. 1997, DAT & al. 1998b, LOPEZ-DELGADO & al. 1998), while conversely SA accumulation can be induced by elevated H_2O_2 levels (CHAMNONGPOL & al. 1998). The mechanisms of these interactions, and their significance in stress signaling, are still incompletely understood.

The hypersensitive response to pathogens exhibits an early 'oxidative burst' of superoxide which rapidly dismutates to H_2O_2. This mechanism involves key interactive roles for SA and H_2O_2, as the hypersensitive response was impaired in tobacco plants with an H_2O_2-inducible SA-hydroxylase transgene (MUR & al. 1997). The 'oxidative burst' enzyme in plants is believed to be a homologue of a mammalian plasma membrane NADPH oxidase (KELLER & al. 1998), and it may be that this enzyme is potentiated by SA (KAUSS & JEBLICK 1996).

Enhanced generation of active oxygen species is common in plants during abiotic stress, and is referred to as 'oxidative stress' because of the potential for cell damage (PRASAD & al. 1994, FOYER & al. 1997, BANZET & al. 1998, DAT & al. 1998b). Many stresses limit CO_2 assimilation more than electron transport capacity leading to modulation of the latter by photosynthetic control. This favours increased oxidation of photosystem I and increased reduction of photosystem II. In these circumstances, electron flow to oxygen is increased yielding superoxide in the Mehler reaction, etc. Alternatively, H_2O_2 generated under high light can originate from photorespiration. In view of recent progress on the oxidative burst in pathogenesis, the possibility of a further, signal-mechanism origin for H_2O_2 in abiotic stress also needs consideration (FOYER & al. 1997). DOKE & al. 1994 first indicated that the NADPH oxidase could be activated by abiotic stresses as well as pathogens. As discussed above, SA can increase during abiotic stress, and so could mediate increased H_2O_2, presumably as a signal mechanism.

The concept of H_2O_2 as a signal is realistic because this molecule is relatively stable and diffusible (FOYER & al. 1997). Effects of exogenous applications of H_2O_2 are consistent with such a signaling role in abiotic stresses, though the number of studies is still small. PRASAD & al. 1994 showed that pre-treatment with 0.1 mM H_2O_2 protected 3-day-old seedlings of maize (*Zea mays* L.) against chilling stress, and induced expression of antioxidant enzymes. BANZET &

al. 1998 demonstrated heat-shock protein accumulation in tomato cells treated with at least 1 mM H_2O_2. LOPEZ-DELGADO & al. 1998 treated nodal explants of potato microplants with H_2O_2 for 1 h during subculture. Over the following month, the microplants pre-treated with 0.1-1 mM H_2O_2 showed greater tolerance of heat-shock. The basis of such long-lived effects of H_2O_2 on growth and thermotolerance is not known; one possibility is an influence on DNA methylation, which can be affected by active oxygen species (CERDA & WEITZMAN 1997).

While the complexity of oxidant physiology is becoming ever more apparent, the benefits for plant protection of improved understanding are enormous.

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