Salicylic Acid and Jasmonic Acid

Biosynthesis, Functions and Role in Plant Development

Phyllis Santos
Editor

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BIOSYNTHESIS, FUNCTIONS AND ROLE IN PLANT DEVELOPMENT

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PHYLLIS SANTOS
EDITOR

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Chapter 2

Induction by Salicylic Acid of *In Vitro* Thermotolerance during Thermotherapy

H. A. López-Delgado, [*,1] *M. Aguilar-Camacho*[1]*,*
R. Martínez-Gutiérrez,[1] *M. E. Mora-Herrera*[2]
and G. Rogel-Millan[2]
[1] Programa de Papa, Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP). Conjunto SEDAGRO, México
[2] Universidad Autónoma del Estado de México, Centro Universitario Tenancingo, Universidad Autónoma del Estado de México, México

Abstract

Virus are an important problem in plants, since they can generate important economic lost in many crops. Production of virus-free plants is a requirement in seed production schemes. This chapter demonstrates the potential effect of salicylic acid on getting virus-free plants. Thermotherapy followed by meristem isolation and culture is the usual method for eradication of virus. Thermotherapy can be done *in vitro* or in pots. The thermotherapy treatment depends on the virus present in the plants and the sensitivity of the cultivar to heat. Often plants do not

[*] lopez.humberto@inifap.gob.mx.

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tolerate the high temperatures for periods long enough to inactivate the virus. Enhanced thermotholerance effect of salicylic acid is demonstrated for getting PVX-free plants in *Solanum tuberosum* and TSWV, TAV-free plants in *Dendranthema grandiflora* Tzvelev. Short and long terms effects of SA were tested in microplants infected. Survival after thermotherapy, recovery period and virus-free microplants were assessed. Both short and long term SA treatments showed a significant survival during thermotherapy. The number of virus-free microplants was also increased by SA treatments in contrast with control. SA was able to improve the survival and the virus-free microplantas. The induction of thermotolerance by salicylic acid is associated with active oxygen species.

1. Main Techniques for Virus Cleaning

Virus diseases are an important limiting factor for plant development and production (González-Pasayo and Huarte, 2011). The production of healthy plants, in terms of viruses, could only be guaranteed by carefully selecting the mother plants that were virus-free by diagnostic protocols followed by propagating those negative plants (Panattoni et al., 2013).

Many techniques have been developed to get virus-free plants from infected individuals. The most regular techniques used are: meristem culture, chemotherapy, cryotherapy electrotherapy and thermotherapy (Mellor and Stace-Smith, 1977; Zapata et al., 1995, Wang and Valkonen, 2008, Lozoya-Saldaña and Dawson, 1982; Panattoni et al., 2013)

In infected plants, viruses tend to be absent from meristematic tissues and young primordial leaves (López-Delgado et al., 2004), meristem culture requires isolation of the apical meristem or from the axillary buds from infected plants and subculture them on a culture medium (Mellor and Stace-Smith, 1977). Meristem culture requires expertise and do not always develop into rooted plants. Chemotherapy involves application of chemicals by either two methods, applied to the infected plant or incorporated into the nutrient medium to decrease the virus concentration (Mellor and Stace-Smith, 1977; Sánchez et al., 1991; Valenzuela-Herrera et al., 2003). Cryotherapy comprises the excision of shoot tips and frozen them in liquid nitrogen, followed by thawing and post-culture in order to regenerate them to plants (Wang and Valkonen, 2008). Electrotherapy is based in the application of electric charges either directly to the plant or indirectly (Lozoya-Saldaña et al., 1986).

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Thermotherapy is a known method which is based in the application of high temperatures in virus–infected plants to retard or inhibit the replication of some viruses (Mellor and Stace–Smith, 1977; Sánchez et al., 1991; Zapata et al., 1995). The aim of thermotherapy consists in getting progressively a less adequate cellular environment for virus replication, but without damaging irreversibly plant tissues (Panattoni et al., 2013).

2. Limiting Factors in Thermotherapy

The temperature and the time of thermotherapy applied depend on the virus present and the sensitivity of cultivar to heat (López–Delgado et al., 2004). The temperature chosen for thermotherapy is a compromise between plant survival and virus elimination (Stein et al., 1991).

Nevertheless, often plants are not able to tolerate their exposure to high temperatures for periods long enough to inactivate the virus replication (Lozoya–Saldaña and Dawson, 1982), also, some cultivars are very sensible to heat treatment which makes difficult obtaining virus–free plants (González–Pasayo and Huarte, 2011). Consequently, yield of virus–free materials depends on the sort of the treatment, sensitivity of cultivar to heat and the virus–host interaction (Faccioli and Colombarini, 1996).

Another limiting factor of thermotherapy is the age of explants. Thermotherapy procedures should allow excised explants to recover before heat treatment; also the older the explants, the higher survival during heat–shock treatment (López–Delgado et al., 1998).

Due to plants often do not tolerate high temperatures before the inactivation of virus replication, an alternative is the application of alternating temperature regimes between supraoptimal and optimal which the plant can tolerate (Lozoya–Saldaña and Dawson, 1982). Alternating temperature regimes during thermotherapy involves incubation of plant explants at high temperatures long enough to temporally inactivate virus replication followed by an optimal temperature in order to permit the plant to recover of this severe stress, plants grew from meristems of this plants could be virus–free (Lozoya–Saldaña and Dawson, 1982). Table 1 shows the use of thermotherapy regimes in different plant species for obtaining virus–free plants.

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Table 1. Plants species subjected to thermotherapy (table modified of *Panattoni* et al., 2013)

Plant Specie	Virus	Temperature Regimes	Authors
Prunus armeniaca, cv. Bebecou	*Plum pox virus*	30-35 C	Koubouris et al., 2007
L. x elegans Thunb	*Lily symptomless virus*	35 ± 1 C	Nesi et al., 2009
Malus domestic cv. Idared and Sampion	*Apple chlorotic leaf spot virus* and *Apple stem pitting virus*	39 ± 0.5 C	Paprstein et al., 2008
Chrysanthemum morifolium cv. Regol Time	*Chrsysantemum B carlavirus*	38 C	Ram et al., 2005
Pyrus pyrifolia cv. Huanghua	*Apple stem grooving virus* and *Apple chlorotic leaf spot virus*	37 C	Wang et al., 2006
Solanum tuberosum L.	*Potato virus X, Potato virus Y, Potato leaf roll virus, Potato virus S*	25 – 45 C	López-Delgado et al., 2004; González-Pasayo and Huarte, 2011; Lozoya-Saldaña and Dawson 1982.
Vitis berlandieri × Vitis riparia Kober 5BB	*Grapevine vitivirus A (GVA), Grapevine fanleaf nepovirus (GFLV), Grapevine fleck maculavirus (GFkV), Grapevine leafroll ampelovirus 1 (GLRaV-1) and Grapevine leafroll ampelovirus 3 (GLRaV-3)*	37 ± 0.5 C	Panattoni and Triolo, 2010
Prunus persica	*Prunus necrotic ring spot virus (PNRSV)*	32 C	Zilkah et al., 2001
Prunus armeniaca	*Plum pox virus (PPV)*	20/37/24 C	Polak and Hauptmanova, 2009
Allium sativum	*Potyvirus*	32/36/38 C	Ramírez-Malagón et al., 2006

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Table 1. (Continued)

Plant Specie	Virus	Temperature Regimes	Authors
Cynara scolymus	*Potyvirus*	28/34 C	Navacchi et al., 2005
Ipomea batatas	Sweet potato feathery mottle virus (SPFMV)	37/39 C	Jeeva et al., 2004

3. SA and Stress Tolerance

3.1. SA in Biotic and Abiotic Stress

Salicylic acid is one phenolic compounds bearing a hydroxyl group or its derivative that are synthesized by plants (An and Mou, 2011; Vlot et al., 2009). This plant growth regulator mediates processes such as: hypersensitive response, thermogenesis, seed germination, photosynthesis, vegetative growth, respiration, flower formation, seed production and others (An and Mou, 2011; Rivas–San Vicente and Plasencia, 2011). This molecule is able to mediate the antioxidant system efficiency in plants (Hayat et al., 2010).

SA is considered one key component on defense signal transduction, is involved in local and endemic resistance to pathogens in plants by inducing different systemic acquired resistance genes (Hayat et al., 2010; Radwan et al., 2008).

SA is know to activate PRP (pathogenic related proteins) expression by increasing the levels of hydrogen peroxide (H_2O_2) and other reactive oxygen species (ROS), which could play a role as second messenger in the defense signaling pathway (Klessig et al., 2000). Besides activation of defense signal transduction, SA was demonstrated to increase the efficiency of the antioxidant system in plants (Hayat et al., 2010).

External application of SA can provide protection against several types of stresses such as: long term drought in *Ctenanthe setosa* (Kadioglu et al., 2011); heavy metal (Krantev et al., 2008; Jing et al., 2007); heat on different plants species (Shi et al., 2006; He et al., 2005; Senaratna et al., 2000; Dat et al., 2000; López–Delgado et al., 1998).

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3.2. SA and Heat Tolerance

3.2.1. SA and Heat Shock Proteins

Heat stress is considered as one of the major stress in crops; plants respond to this kind of stress through different mechanisms, modifying enzymes activity, cellular membrane structure, photosynthesis activity, protein metabolism and more (Singla et al., 1997). Studies in *Oriza sativa* demonstrated changes in nucleus membrane, endoplasmic reticulum, mitochondria and chloroplasts under heat stress (Pareek et al., 1998).

Response to heat stress at molecular level is found in all organisms, especially sudden changes in genotypic expression, resulting in an increase of some groups of protein synthesis. These groups are called heat-shock proteins (HSPs). These proteins are grouped into 5 classes according to their molecular weight: HSP100, HSP90, HSP70, HSP60 and small heat-shock proteins (sHSP).The higher plants can produce 20 to 40 types of HSPs as adaptation to different types of stress (Al-Whaibi, 2011).

Transcription of heat-shock protein genes is controlled by regulatory proteins called heat stress transcription factors (Hsfs). Plants present at least 21 Hsfs each one having a role in regulation and also cooperating in all phases of heat stress responses (triggering, maintenance and recovery) (Al-Whaibi, 2011).

On the other hand, plants previously treated with SA increased survival to heat stress (López-Delgado et al., 1998; Pavlova et al., 2009; Snyman and Cronjé, 2008), and it has been reported that SA is involved in HSPs regulation. SA is known to stabilize the trimmers of heat-sock transcription factors and to aid in binding to the heat-sock element in the promoter of HSP genes (Jurivich et al., 1992).

It was suggested that SA *per se* did not favored HSPs synthesis without any stress; however, SA augmented the induction of these proteins when plants were exposed to severe heat-shock (Snyman and Cronjé, 2008). Pavlova et al. (2009) demonstrated that at room temperature, SA did not induce synthesis of specific heat-shock proteins HSP101, HSP60 y HSP17.6; however; moderate heat-shock (37 C) induced the expression of HSP101 and HSP17.6 in *A. thaliana* cells. In tomato seedlings SA alone led to activation of Hsf–DNA binding, but not to induction or transcription of *hsp70* mRNA, heat–shocked Hsf–DNA binding was established, and increased *hsfA1*, *hsfA2*, and *hsfB1* expression was followed by accumulation of HSP70. SA and heat shock enhanced Hsf–DNA binding, induction of *hsp70* mRNA transcription, and gene expression of *hsfA1*, *hsfA2*, and *hsfB1*, resulting in potentiated levels of

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Hsp/Hsc70. SA–mediated potentiation of HSP70 due to modulation of Hsfs (Snyman and Cronjé, 2008).

3.2.2. SA and Heat Associated to Oxidative Stress

There is evidence of diverse interactions between SA and production of reactive oxygen species (ROS) meanly H_2O_2, in the host plants signaling during pathogenesis (Chen et al., 1993; Chamnongpol et al., 1996; Vlot et al., 2009) and for H_2O_2 production during biotic and abiotic stresses associated to signaling mechanisms (Foyer et al., 1997). Extreme temperatures, high light, drought, various pollutants involve H_2O_2 production (Foyer et al., 1997; Hayat et al., 2010).

The antioxidant enzyme catalase scavenges excessive H_2O_2 accumulation, which it is potentially harmful to plant cell. Catalase has been suggested as a target of SA action (Chen et al., 1993, Conrath et al., 1995). Potato nodal explants subcultured on to acetyl salicylic acid (ASA)–free medium following several weeks of growth on ASA were more thermotolerant (by 3.8 fold) of a 7 weeks 35 C heat treatment, and (by 38–fold) of a 15 h 42 C heat– shock. Stems of microplants grown on ASA contained significantly less catalase activity and higher levels of H_2O_2 than controls (López–Delgado et al., 1998). Explanting and heat treatment reduced catalase activity to similar levels in ASA–treated and control nodal cuttings. The results suggested that both ASA and H_2O_2 can induce thermotolerance. SA and ASA can bind to and inhibit the catalase activity in tobacco (Chen et al., 1993), this leads to a higher endogenous levels of H_2O_2 on stems of microplants cultured on ASA medium. The role of H_2O_2 as signal could explain the induction of thermotolerance by ASA in potato nodal explants (López–Delgado et al., 1998). The role of H_2O_2 in the signal transduction during the induction of thermotolerance was confirmed. Nodal explants were incubated for 1h in H_2O_2 (0.1–50mM), and then cultured without the H_2O_2. Microplants that grew from these explants were significantly more tolerant than controls (López–Delgado et al., 1998).

Enhanced procedures for thermotherapy of microplants would be useful in potato biotechnology. The thermotherapy treatment depends on the virus present in the plants and the sensitivity of the cultivar to heat. Often potato plants do not tolerate thermotherapy treatments long enough for virus inactivation. SA is known to play an important role in plant defense responses to viral infection (Takahashi et al., 2002; Vlot et al., 2009). Considering the thermotolerance effect induced by ASA in potato nodal explants during heat shock (López–Delgado et al., 1998), we tested SA in thermotherapy (López–Delgado et al., 2004), bearing in mind literature suggesting similar effects for

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appearance to the healthy ones; similar effects were obtained in *Vicia faba* (Radwan et al., 2008) spraying with SA prior inoculation with bean yellow mosaic virus (BYMV) as well as an increased resistance against the virus infection. Similar reports demonstrated induction of resistance by SA against different viruses, as PVX in tomato (Falcioni et al., 2014), peanut mottle virus in *Arachis hypogaea* (Kobeasy et al., 2011).

These papers demonstrated the capacity of SA in inducing resistance against virus infections, alleviating negative effects of virus.

3.4. SA and Virus Cleaning by Thermotherapy

SA induced thermotolerance during thermotherapy at 42 C but also improved the number of PVX–free potato microplants obtained (López–Delgado et al., 2004). We had demonstrated that the concentration 10^{-5} M of SA added into the nutrient media is capable to induce thermotolerance in PVX–infected potato microplants during thermotherapy (42 C, 30d), moreover, SA was able to improve the obtaining of PVX–free microplants. We concluded these effects in thermotolerance and increased percentage of virus–free plants were due to an increase in H_2O_2 internal concentration and a decreased CAT activity.

We also carried out some experiments to determine if SA could induce similar effects in other plant species. We treated with SA *Dendranthema grandiflora* cv. Polaris white microplants infected with Tomato Spotted Wilt Tospovirus (TSWV), Chrysanthemum Aspermy Cucumovirus (TAV) or a mixture of both viruses (Table 2). SA treatment improved thermotherapy and recovery survival after heat treatment (37 C, 25–30 d). In addition, SA enhanced the number of virus–free microplants. These results are similar to those obtained by López–Delgado et al. (2004).

In spite of the generated knowledge about the effects of SA in inducing thermotolerance, few are known about the long term application of this molecule. Experiments with PVX–infected potato microplants clone 040138 were conducted to confirm if SA (10^{-5} or 10^{-6} M) triggers a long term effect in inducing thermotolerance and obtaining virus–free plants. Both concentrations of SA were efficient in inducing thermotolerance and increasing the percentage of PVX–free microplants. Although recovery survival was not enhanced in SA treatments compared with control, thermotherapy survival and PVX–free microplants were enhanced by both SA treatments (Table 2).

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both salicylates on induction of thermotolerance (López-Delgado et al., 1998; Dat et al., 2000). SA appeared to offer a double benefit as a medium supplement in potato tissue culture thermotherapy, enhancing both microplant survival and virus eradication rates. The enhanced survival rate was especially pronounced in cultivars intolerant to thermotherapy. SA treated plants reduced catalase activity associated with increased H_2O_2 levels (López-Delgado et al., 2004). The interaction between salicylates, antioxidants, H_2O_2 and virus is strong justification for continued investigation.

3.3. SA and Its Interaction with Virus Infections

Signaling the activation of disease resistance by SA following pathogen infection has been reported (Vlot et al., 2009). Exogenous application of SA can induce resistance to viruses, even in plants lacking resistance gene. Resistance is described by a decreased virus yield and a delay in the onset of disease symptoms (Murphy et al., 1999). It had been demonstrated that SA is able to inhibit the replication or cell to cell movement of some viruses. Furthermore, it was reported that SA inhibited RNA accumulation of potato virus X (PVX), tobacco mosaic virus (TMV) and alfalfa mosaic virus (AlMV) in SA-treated tissue (Naylor et al. 1998; Chivasa et al., 1997; Hooft van Huijsduijen et al., 1986). Despite SA did not inhibited cucumber mosaic virus (CMV) RNA in tobacco, it delayed virus movement out of the inoculated tissue (Naylor et al., 1998). These reports demonstrated SA can induce interference with virus accumulation at the point of inoculation and also induce inhibition of virus movement out of inoculated tissue. However, among viruses there might well be varying degrees of sensitivity to the effects of SA (Murphy et al., 1999).

Singh et al. (2004) suggest that SA triggers resistance to three different phases of the viral infection process: replication, cell-to-cell movement and long-distance movement. Even though a particular virus may be able to evade the SA-induced inhibition of virus replication, it may be affected by other defensive response.

Due to SA is involved in the induction of resistance against virus infections, research had been carried out to determine the effects of SA in different virus-infected plants.

Radwan et al. (2006) sprayed *Cucurbita pepo* leaves with SA before inoculation with zucchini yellow mosaic virus (ZYMV) which allowed to recovery from the undesirable effects induced by this virus, and with alike

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Table 2. Effects of SA in virus cleaning by thermotherapy. LT: Long term, ST: Short term; N.D: No data

Plant Specie	Virus	SA[M]		Temperature Regime		Thermotherapy Period		Survival (%)		Recovery (%)		Virus-Free Plants (%)	
		ST	LT	ST	LT	ST	LT	ST	LT	ST	LT	ST	LT
Dendranthema grandiflora cv. Polaris white	*Tomato Spotted Wilt Tospovirus* virus (TSWV)	10^{-5}	N.D	37 C	N.D	25–30 d	N.D	30.00	N.D	100	N.D	100	N.D
		0						15.00		100		0	
	Chrysanthemum Aspermy Cucumovirus (TAV)	10^{-5}						81.82		0		N.D	
		0						75.76		0		N.D	
	TSWV y TAV	10^{-5}						60.63		100		100	
		0						50.00		100		0	
Solanum tuberosum L. clone 040138	Potato Virus X	10^{-5}	10^{-5}	32 C/22h –42 C/2h		30d		80.00	100	86.84	63.33	30.45	32.4
		10^{-6}	10^{-6}					76.67	100	75.83	43.33	38.09	38.09
		0	0					40.00	63.53	87.76	63.53	19.16	20.20

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Conclusion

SA has proven to be a potent natural compound for inducing stress tolerance either against heat treatment or virus infections. The potential application of SA to virus cleaning technics accompanied with thermotherapy, can improve the virus-free material obtained. Further research about the effects of SA on other plant species is matter of confirmation.

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