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Comparative of some growth, biochemical, and hormonal responses of galled branches of two species of willow trees

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Abstract
The witches' broom of the willow trees is induced by photoplasma in the galled branches. The aim of the study is the evaluate the changes in growth and physiological factors and the plant growth-promoting hormones (Auxin, Gibberellin, and Cytokinin) in leaf witches' broom branches compared with those healthy symptomless branches. The samples were collected from the leaves of ungalled and galled branches of both willow trees in Najvan Park, Isfahan. Results showed that growth parameters, total chlorophyll, and total soluble carbohydrates were decreased in the leaves of witches' broom branches in both species. However, the relative water content and polyphenol oxidase activity did not show a significant change as compared with those of ungalled branches. In contrast, the ascorbate peroxidase activity did not show a significant difference between the leaves of ungalled and witches' broom branches in S. babylonica, but its activity was decreased in witches' broom branches of S. alba. The cytokinin content was increased (more than 50%) in the leaves of witches' broom branches of both species, but the auxin and GA_3 contents in the leaves of witches' broom branches of S. alba and S. babylonica were significantly decreased by 30.6% and 33.6% respectively as compared with those of healthy control leaves. The concentration of total chlorophyll and water-soluble sugar decreased due to oxidative stress induced by phytoplasma. Reduced growth with no significant change in the relative water content of galled branches in both willow trees accompanied by the specific hormonal changes of each species which is formed of specific galls for each species.

Introduction
The genus Willows (Salix) are shrubs and trees that usually grow in most parts of the earth. Plant galls are neoformed organs that develop under the influence of galling organisms. Insects, nematodes, viruses, bacteria, mycoplasma, fungi, other plants, and mites are typically considered gall inducers. They cause structural and metabolic changes in their host plants, leading to the formation of the gall organ. Three hypotheses nutrition, microenvironment, and enemy have been proposed for forming galls by

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insects. The gall formation depends on the level of gene transcription interactions that exist between gall inducers and plants. The function of these genes causes changes in growth and development processes, cytoskeleton organization, cell walls, metabolic pathways, and plant hormone pathways. Recently, *Candidatus* phytoplasma trifolii caused willow catkin gall on *Salix babylonica* and *S. alba* trees, characterized by a unique case of neoplastic formation like witches' broom structure. The aim of the study is the evaluate of the changes in growth and physiological factors and the plant growth-promoting hormones (Auxin, Gibberellin, and Cytokinin) in leaf witches' broom branches compared with those healthy symptomless branches.

Material and methods

Leaf samples of trees with gall and their symptomless adjacent branches were collected from Najvan Park, Isfahan province, Iran. The length and width of the leaves were measured and recorded. Fresh and dry weights and relative water content (RWC) of leaves were measured. Total chlorophyll was measured according to the Arnon assay. Water soluble carbohydrates were assessed using the method of Dubois et al. (1956). Activities of ascorbate peroxidase (APX) and polyphenol oxidase (PPO) were measured by the methods of Asada and Takahashi (1987) and Kar & Mishra (1976), respectively. High-performance liquid chromatography (HPLC) was used to analyze plant growth-promoting hormones.

Results and discussion

The gall formation in *Salix* species is caused by *Candidatus* phytoplasma trifolii. The gall formation was accompanied by a reduction in the growth of leaves and shoots, which was represented by smaller leaves and shorter internodes. The gallers used nutritional resources of gall and adverse effects on the growth and survival of the host plants Results showed that relative water content (RWC) showed no significant changes between the witches' broom structure compared with those of healthy control leaves in both *Salix* species. Reduction in growth without change in RWC is due to the modification in the expression of genes which is indicated by smaller leaves in gall. Total chlorophyll was reduced in all gall-infected tissue of both *Salix* species. The reactive oxygen species (ROS) generated to respond to the biological stress in chloroplast can induce oxidative damage to the photosynthetic pigments which leads to the reduction of soluble sugars in the galled leaves. Polyphenol oxidase (PPO) activity did not show a significant change as compared with those of healthy branches in both galled leaves *Salix* species. In contrast, the ascorbate peroxidase activity did not show a significant difference between the leaves of ungalled and galled branches in *S. babylonica*. However, its activity was decreased in galled branches of *S. alba*, which indicated the activity of these enzymes is not a priority in both galled leaves *Salix* species. The cytokinin content was increased in the leaves of witches' broom branches of both species. However, the auxin and GA_3 contents in the leaves of witches' broom branches of *S. alba* and *S. babylonica* were significantly decreased by 30.6% and 33.6% respectively as compared with those of healthy control leaves. Enhanced cytokinin content in both willow species can be introduced as the main factor in increasing the divisions of witches' broom branches, which is coordinated with the particular hormonal changes each species which generates special galls them.

Conclusions

Candidatus phytoplasma trifolii reduced the growth of infected tissues characterized by smaller leaves and shorter internodes, followed by the formation of gall structure in both Salix species. According to the results, the high accumulation of ROS in response to the gall formation was accompanied by a reduction of total chlorophyll and water-soluble carbohydrates. Altogether, it seems that the adverse effects of gallers on Salix species refer partly to oxidative stress, which is synchronized with the specific hormonal changes of each species. These events cause the creation of special galls for each species.

Keywords: Enzymes activity, Gall, Growth promoting hormones, Total chlorophyll, Willow

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(Guiget et al., 2021;

(Mohajer Kazemi et al., 2020) (Salix spp.)

(Tawfeek et al.

2003; Stone & Schönrogge 2021;

et al., 2022; Bajraktari

et al., 2019; Cambier et al., 2023; Salehi-Eskandari

Takeda et al., 2019 (S. alba)

et al.; Schultz

et al., 2019; Takeda 2019 1984

, 2014; Khajuria et al. Bohlmann & Sobczak

Gheysen & al., 2023

(Favery et al., 2020; Guiget et al., 2021;

al., 2019; Mitchum Oliveira et al., 2016).

(microenvironment)

(enemy)

Salehi-

, 2015; Eskandari & Kaviani

, 2003; Stone & Schönrogge

Rhabdophaga

(Samsone et al., 2011)

et al., 2020; Harris et al., 2006; Favery

et al., 2016; Oliveira

(Salix alba)

Nyman &

(S. alba, S. babylonica) , 2000; Salehi-Eskandari & Julkunen-Tiitto

Kavian, 2015

(Willow (Willow catkin gall)

(witches' broom)

(Candidatus

Khadhair & phytoplasma arifolii)

(S. babylonica) (S. alba) (S.Babylonica) , 1995 Hiruki

, 2011 Shahryari & Allahverdipour

2018 Ghayeb Zamharir (S. alba)

(S. babylonica) (S. alba)

(C, D)

Figure 1- Picture of willow trees, Salix alba (right side) and Salix babylonica (left side) which one of their galls branches is shown at the bottom of the pictures (C, D)

Eskandari et al., 2017

pH= 7.8

DDT

EDTA

(Relative Water Content; RWC) (Kreslavski et al. 2022)

$$RWC = \frac{FW - DW}{DW} \times 100$$

(Ascorbate peroxidase, APX)

$mM^{-1}\ cm^{-1}$

Asada & Takahashi, 1987

(polyphenol oxidase, PPO)

Arnon 1949

Shimadzu model UV-160

Kar & Mishra, 1976

$$Chl\ T(mg/g) = (20.2A_{645} + 8.02A_{663}) \times \frac{V}{1000} \times \frac{1}{W}$$

Bradford, 1976

SAS 9.1

LSD

Beijing Karaltay

Salehi

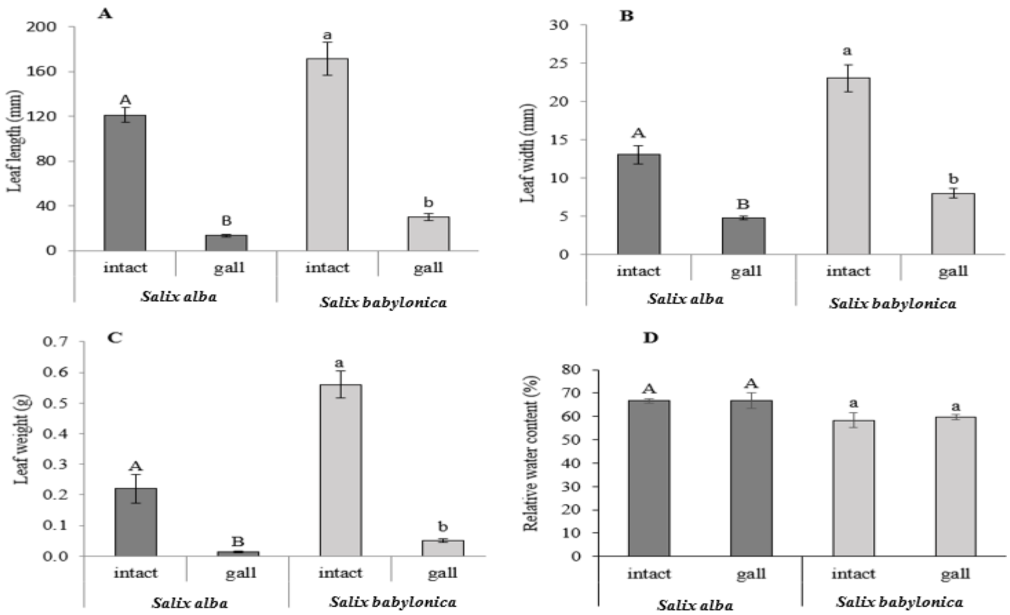

Figure 2- Comparison of average length(A), width (B), weight (C) and relative water content of leaves (D) of ungalled and galled branches on two species of Salix. Values are expressed by the mean ± standard error. In each species, different letters (uppercase for S. alba and lowercase for S. babylonica) indicate statistically significant differences in t-test ($P < 0.05$).

P < 0.05

S. babylonica

S. alba

S. babylonica

S. alba

t-test

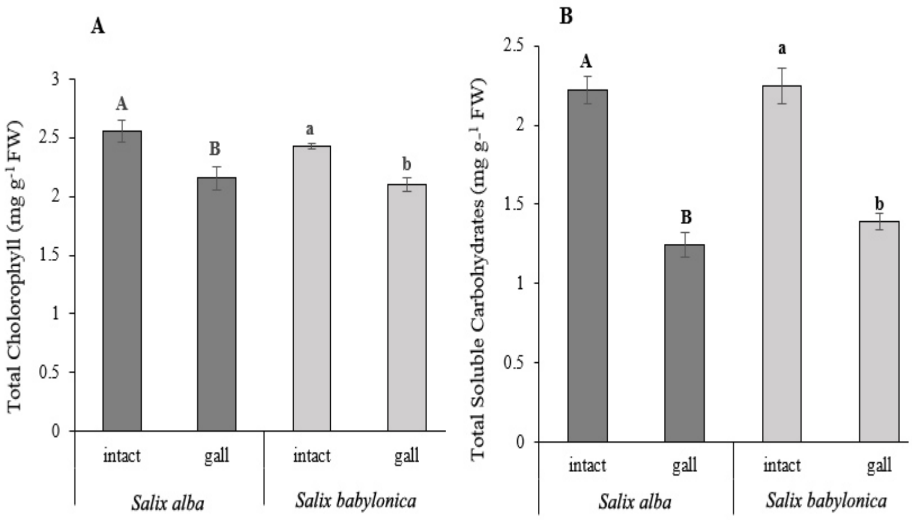

Figure 3- Comparison of average total chlorophyll (A) and total soluble carbohydrates (B) of ungalled and galled branches on two species of Salix. Values are expressed by the mean ± standard error. In each species, different letters (lowercase for S. alba and uppercase for S. babylonica) indicate statistically significant differences in t-test (P < 0.05).

P >

(PPO)

0.05

(AAX)

P > 0.05

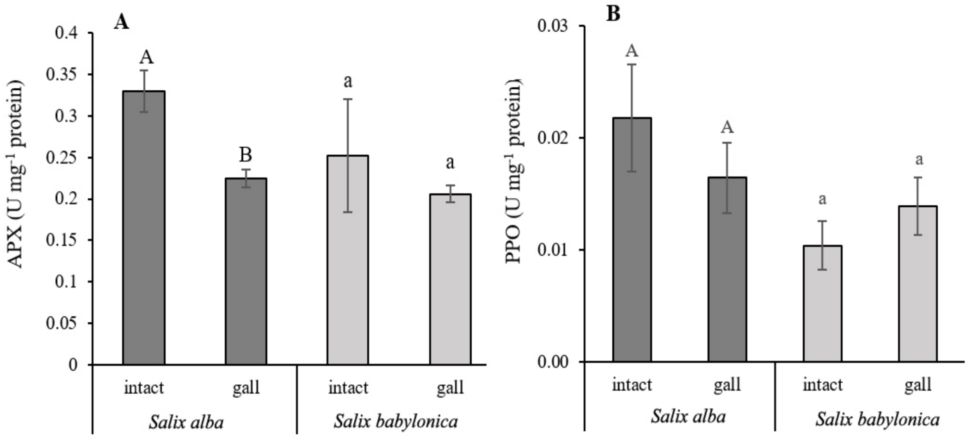

Figure 4- Comparison of the average activity of enzymes ascorbate peroxidase (APX) (A) and polyphenol oxidase (PPO) activity of leaves (B) of ungalled and galled branches on two species of *Salix*. Values are expressed by the mean ± standard error. In each species, different letters (lowercase for *S. alba* and uppercase for *S. babylonica*) indicate statistically significant differences in t-test ($P < 0.05$).

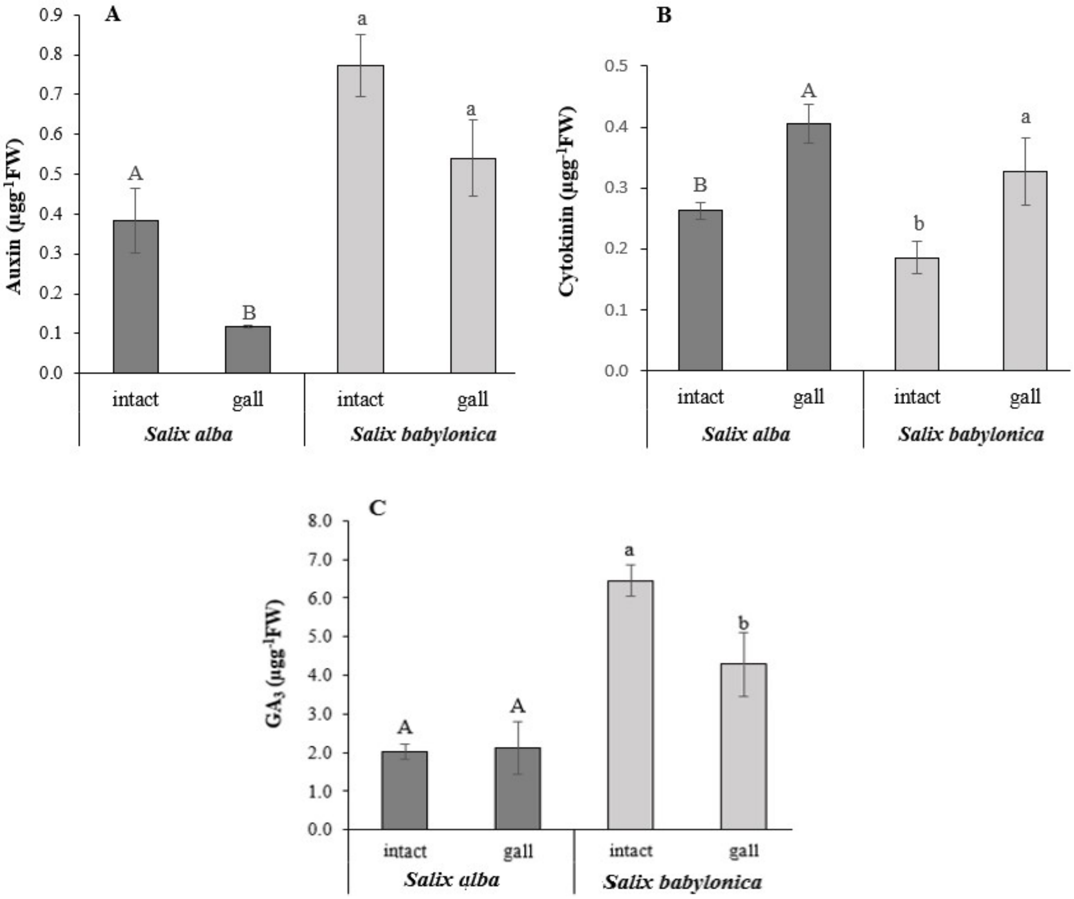

Figure 5- Comparison of average the hormones auxin (A), cytokinin (B) and gibberellin (C) of ungalled and galled branches on two species of Salix. Values are expressed by the mean ± standard error. In each species, different letters (lowercase for S. alba and uppercase for S. babylonica) indicate statistically significant differences in t-test (P < 0.05).

et al., Nyman

2000; Saleh-Eskandari & Kaviani, 2015

Candidatus

Ghayeb Zamharir phytoplasma trifolii"

2018; Shahryari & Allahverdipour, 2018

et al., 2016 Giron

et al., 2007; Salehi-Eskandari et Murata et Stone

Gailite et al., 2024 et al., 2002

et al., 2005 (RWC)

Picea abies, *Acer sacharinum*, *Ulmus laevis*, *Ulmus glabra*, *Tilia cordata*

(*Salix* (Pratama et al., 2013)

pulchra, *Salix glauca*),

et al., 2019; Favery et al., 2016; Cambier

(Crown gall)

Agrobacterium tumefaciens

et al., 2014; Huang

DNA

B

et al., 2019; Kerpen

et al., 2022; Ivanova

(*Salix*

Salehi-Eskandari & (Ebba)

, 2015; Kavhiza

(reactive oxygen species;

(*Tilia* (Asada, 1999) (ROS)

(*Eriophyes tiliae* (*platyphyllos*)

et al., 2023; Guedes

et al., 2022; Sherin

et al., 2019; Samsone

(S. alba, S.

(S. babylonica), et Kot

et al., 2020

(S. caprea), Barna & Pogány

et Samsone, 2001; Kotel et al., 2020

et al., 2019

(S. fragilis)

et al., 2006; Gailite, Rashtizadeh, Rahnemi &

Samsone

Kmieć

2022

H_2O_2

et al., 2020; Pavery

(Waruta et al., 2016)

et al., 2016; Giron

E. T. acaciaelongifoliae

solidaginis

et al., 2005; Gailite

., 2014; Tooker & Helms

[illegible] et al., 2020; Favery [illegible] Kmieć et al., 2012 [illegible] et al., 2013 [illegible]

[illegible] *babylonica* [illegible]

[illegible] (Tooker & Helms, 2014) [illegible]

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