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超敏蛋白对低温胁迫下核桃生理特性的影响

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摘 要:【目的】研究超敏蛋白对低温胁迫下核桃生理特性的影响,探讨其提高核桃抗寒性可能的作用机制,为超敏蛋白在核桃抗逆生产中的应用提供理论依据。**【方法】**以抗寒力较弱的早实核桃品种'香玲'为试材,采用大田喷施与室内模拟低温环境处理相结合的方法,测定低温(2、0、-2、-4、-6 ℃)胁迫下核桃叶片的相对电导率、丙二醛(MDA)含量、可溶性蛋白含量、可溶性糖含量、脯氨酸含量及超氧化物歧化酶(SOD)、过氧化物酶(POD)和过氧化氢酶(CAT)活性,探究超敏蛋白在核桃响应低温胁迫中的作用。**【结果】**随着温度的降低,核桃叶片相对电导率、丙二醛含量、可溶性蛋白含量、脯氨酸含量及超氧化物歧化酶(SOD)、过氧化物酶(POD)、过氧化氢酶(CAT)活性均升高,可溶性糖含量降低;喷施超敏蛋白可显著降低0 ℃以下低温胁迫核桃叶片的相对电导率、丙二醛含量,显著提高可溶性蛋白含量、脯氨酸含量和超氧化物歧化酶(SOD)、过氧化物酶(POD)、过氧化氢酶(CAT)活性,对可溶性糖含量影响不大。**【结论】**喷施超敏蛋白可以提高低温胁迫下核桃叶片脯氨酸含量和可溶性蛋白含量,增强抗氧化酶活性,降低相对电导率和MDA含量,以维持细胞正常的生理代谢,防止膜脂过氧化和保护细胞膜结构的稳定性,增强核桃的抗寒性。

关键词:核桃;超敏蛋白;低温胁迫;生理指标

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Effect of harpin on physiological characteristics of walnut under low temperature stress

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Abstract:【Objective】'Xiangling' is an early fruiting walnut cultivar widely cultivated in north China, but it is susceptible to cold and often damaged by late frost, which results in significant decline in yield or even total crop failure. Harpin is a stable polypeptide isolated from *Erwinia amylovory*, and has been used as a pure biopesticide that induce resistance in plants. In this study, the effect of harpin on physiological characteristics of walnut under low temperature stress was studied in order to explore the mechanism of harpin improving cold resistance of walnut and to provide theoretical basis for the application of harpin in walnut production.【Methods】1.5 g·L^{-1} of harpin was sprayed to 6-year-old trees of walnut cultivar 'Xiangling' prior to female flower stage with distilled water spray as the control. The experiment was conducted with a random block design, with 5 trees in each of 3 blocks. 7 d after spraying, shoots of the same diameter and length were sampled from the same direction of the canopy of each treated walnut tree and placed into a phytotron (RXZ-0288)and exposed to different low temperatures (2, 0, -2, -4 and -6 ℃). The temperature in the phytotron was initially set at 10 ℃ and programmed to decrease at a rate of 4 ℃·h^{-1} and maintained at each target low temperature for 8 h, and then the temperature was set to 10 ℃ again. The

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third leaf from the top was taken from the young sprout after 5 h at 10 ℃. The samples were frozen in liquid nitrogen and stored under −70 ℃. The relative electricity conductivity was determined using an electricity conductivity meter. Soluble protein was determined with coomassie brilliant blue. Soluble sugars were determined with anthrone colorimetric method, proline content with acidic−ninhydrin reaction method, and malondialdehyde (MDA) with thiobarbituric acid method. The superoxide dismutase (SOD) activity was determined with the method of Xue Guoxi, peroxidase (POD) with the method of guaiacol, and catalase (CAT) with the method of ultraviolet absorption.【Results】With the decrease of temperature, the relative electricity conductivity and malondialdehyde (MDA) content in walnut leaves increased in both the treatment (sprayed with harpin) and the control (spraying distilled water), but they were lower in the former than in the latter. The relative electricity conductivity in the treatment and the control was 27.71% and 32.13% at −6 ℃, respectively, and their content of MDA was 24.51 μmol·g^{-1} and 18.25 μmol·g^{-1}, respectively. The relative electricity conductivity in the treatment and the control had significant difference under various low temperatures and the MDA content had significant differences under the low temperature of −2, −4 and −6 ℃. The activities of SOD, POD and CAT increased with the decrease in temperature and were higher in the treatment. The activity of SOD in the treatment (119.23 U·g^{-1}) was 1.22 times that of the control (97.71 U·g^{-1}) at −6 ℃; POD activity in the treatment (0.032 μmol·g^{-1}·min^{-1}) was 1.39 times that of the control (0.023 μmol·g^{-1}·min^{-1}); and CAT activity in the treatment (0.124 μmol·g^{-1}·min^{-1}) was 1.36 times that of the control (0.091 μmol·g^{-1}·min^{-1}). The activities of SOD, POD and CAT were significantly different between the treatment and the control under the temperatures of 0, −2, −4 and −6 ℃. The contents of proline and soluble proteins increased with the decrease in temperature, and they were higher in the treatment than in control. Proline content in the treatment (98.85 μg·g^{-1}) was 1.112 times that of the control (88.93 μg·g^{-1}) under the −6 ℃. Soluble protein content in the treatment (12.17 mg·g^{-1}) was 1.166 times that of the control (10.44 mg·g^{-1}). Under the low temperatures of 0, −2, −4 and −6 ℃, the contents of proline and soluble proteins were significantly different between the treatment and the control. With the decrease in temperature, the content of soluble sugars in the treatment and the control decreased, but the content of soluble sugars in the treatment was higher than in the control. When the temperature decreased from 2 to −6 ℃, the percentage of decrease in soluble sugar content in the treatment (11.8%) was lower than in the control (15.2%). But the difference in soluble sugar content between the treatment and the control was not significant.【Conclusion】Spraying harpin increased the contents of proline and soluble proteins and the activities of antioxidant enzymes in the leaves of walnut but reduced the relative electricity conductivity and MDA content under low temperature stress. The treatment helped the walnut to maintain normal physiological status of cells, preventing membrane lipid peroxidation, maintaining the integrity of membrane structure, and enhancing the cold resistance.

Key words: Walnut; Harpin; Low temperature stress; Physiological index

核桃(*Juglans regia* L.)是中国重要的经济林树种，但在北方核桃产区普遍存在倒春寒和晚霜危害，使核桃新梢、雌花和幼果受冻，造成核桃产量大幅度降低甚至绝收[1−3]，严重制约了核桃产业的发展，因此，如何提高核桃新梢生长期的抗寒性已成为核桃生产上亟待解决的问题。

目前利用外源物质(如NO、Ca^{2+}、EBR、H_2S、ABA等)提高植物抗寒性的研究已在核桃、结缕草(*Zoysia japonica*)、玉米(*Zea mays*)、黄瓜(*Cucumis sativus*)、萝卜(*Raphanus sativus*)等多种植物上得到证实[4−8]，并在植物抗低温逆境中起到了重要作用。因此研究外源物质对植物抗寒力的影响，探讨其在植