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Expression of the *Galanthus nivalis* agglutinin (*GNA*) gene in transgenic potato plants confers resistance to aphids

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ABSTRACT

Aphids, the largest group of sap-sucking pests, cause significant yield losses in agricultural crops worldwide every year. The massive use of pesticides to combat this pest causes severe damage to the environment, putting in risk the human health. In this study, transgenic potato plants expressing *Galanthus nivalis* agglutinin (*GNA*) gene were developed using CaMV 35S and ST-LS1 promoters generating six transgenic lines (35S1-35S3 and ST1-ST3 corresponding to the first and second promoter, respectively). Quantitative real-time polymerase chain reaction (qRT-PCR) analysis indicated that the *GNA* gene was expressed in leaves, stems and roots of transgenic plants under the control of the CaMV 35S promoter, while it was only expressed in leaves and stems under the control of the ST-LS1 promoter. The levels of aphid mortality after 5 days of the inoculation in the assessed transgenic lines ranged from 20 to 53.3%. The range of the aphid population in transgenic plants 15 days after inoculation was between 17.0 ± 1.43 (ST2) and 36.6 ± 0.99 (35S3) aphids per plant, which corresponds to 24.9–53.5% of the aphid population in non-transformed plants. The results of our study suggest that GNA expressed in transgenic potato plants confers a potential tolerance to aphid attack, which appears to be an alternative against the use of pesticides in the future.

1. Introduction

Aphids are considered as the largest group of sap-sucking pests that cause significant yield losses in agricultural crops worldwide [1–3]. They induce damage to their host plants, modifying plant metabolism, ingesting plant nutrients from the phloem, and vectoring plant-pathogenic viruses [4,5]. *Macrosiphum euphorbiae* (Thomas), and *Myzus persicae* (Sulzer) aphid species constitute one of the major potato pests affecting the production of this crop [6], which is considered the fourth most important worldwide [7].

Despite the improvement of biopesticides like toxins derived from entomopathogenic fungus [8] and bacteria [9], and spiders venom [10] that have specificity for target pest species [11] and do not affect mammals [12], these substances remain in the epidermis, being effective only for crewing insects. Notwithstanding the fact they combat aphids, which are able to penetrate their long acupuncture mouthparts into plant phloem, it is necessary to search for effective substances that must be able to reach these active sites with a long-time permanence and low digestion ratio

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once it has been ingested by the insect. One candidate substance that complies with the aforementioned requirement is the snowdrop lectin *Galanthus nivalis* agglutinin (GNA), which is able to cross the midgut epithelium [13] remaining stable and active within the insect gut after having been ingested. It has been reported that GNA confers resistance to crewing and sap-sucking insects in rice, tobacco, cotton, rape, and wolfberry [14–16], without toxicity to higher animals [17]. The use of genetic engineering that allows the synthesis of GNA to improve the resistance of important crops like potato to aphids is yet a pending issue that needs to be implemented.

Transgenic technology has allowed the expression of a broad spectrum of promoters, which potentially are involved in the synthesis of lectins, one of them, the CaMV 35S promoter, is most widely used in transgenic plants, since it not only affects the associated transgene, but also exerts influence in thousands of base pairs up- or downstream of the insertion site on a given chromosome [18,19]. However, the foreign gene, driven by CaMV 35S promoter, is expressed in all tissues during plant growth and development [20]. It causes consumption of excessive matter and energy within the cells for the expression of the target gene, affecting its temporal and spatial effectiveness [21]. Furthermore, there are other promoters that are only expressed in photosynthetic tissues; this is the case of ST-LS1, a light-inducible promoter whose expression has been detected in leaves and photosynthetic stems of potato [22–24]. In the present study, the transgenic potato plants expressing *GNA* gene driven by CaMV 35S and ST-LS1 promoters respectively were obtained to find the difference of tissue-specific expression and to estimate the resistance of the transgenic potato plants to aphids. Our aims were to obtain the transgenic potato plants expressing the *GNA* gene driven by CaMV 35S and ST-LS1 promoters, respectively, to find the difference of the tissue-specific expression of the *GNA* gene and further to improve the resistance of potato to aphids.

2. Materials and methods

2.1. Plants and insects

The potato cv. 'Atlantic' was propagated *in vitro* by subculturing single-node cuttings on Murashige and Skoog medium [25] supplemented with 3% sucrose and 0.45% agar. Plantlets were grown in 150-mL flasks under white fluorescent light during 16 h and in the dark during 8 h, at a temperature of 24 ± 2 °C. Microtubers were induced in the dark at 24 ± 2 °C in an MS medium supplemented with 8% sucrose and 0.45% agar [26]. Green peach aphids (*M. persicae*, Sulzer) were collected from Yuzhong County in Gansu Province, China. Aphids were reared in a light incubator at 25 ± 2 °C under a 14 h light/10 h dark photoperiod, and fed with plantlets of potato cv. 'Atlantic'.

2.2. Construction of the plant expression vector

The fragment of *GNA* gene with nucleotide sequence (GenBank accession No. M55556.1) [27] was digested with *Bam*H I and *Sal* I from the clone vector and ligated into a binary vector pBI121 [28] and pBI121-ST-LS1 [24] resulting in the recombinant vectors pBI121-CaMV35S-GNA and pBI121-ST-LS1-GNA. These vectors were transformed into *Escherichia coli* DH5α respectively, and further verified by the same restriction endonuclease digestion. After that, pBI121-CaMV35S-GNA and pBI121-ST-LS1-GNA were transformed into *Agrobacterium tumefaciens* LBA4404, respectively, using the freeze-thaw method [29].

2.3. Transformation of potato

Potato transformation was performed according to the protocol of Si et al. [30]. Microtuber slices of potato cv. 'Atlantic' were co-cultured (media-MS + 1 mg/L IAA + 0.2 mg/L GA_3 + 0.5 mg/L 6-BA + 2 mg/L ZT) for 2 days with *A. tumefaciens* LBA4404 containing the plasmid pBI121-CaMV35S-GNA and pBI121-ST-LS1-GNA, respectively, then transferred into a selection media supplemented with 50 mg/L kanamycin. When green buds sprouted from the surface of the slices and reached a length of 1 cm, they were transferred to a selective rooting medium containing 100 mg/L of kanamycin and 200 mg/L of carbenicillin. Plantlets with well-developed roots were propagated for further molecular analysis.

High-quality DNA was isolated from the leaves of putatively transformed and non-transformed (NT) control potato plants for PCR according to the method proposed by Edwards et al. [31]. The part of the coding sequence of the *GNA* gene was amplified using a PCR Screening Kit (GenStar, Beijing, China) with forward primer (5′-GCGGATCCATGGCTAAGGCAAGTCTCC-3′) and reverse primer (5′-GTACGAGCTCTTACTTTGCCGTCACAAGCT-3′). Amplification was performed in a thermal cycler (T100™, BIO-RAD) programmed for one cycle of 3 min at 94 °C followed by 30 cycles of 30 s at 94 °C, 30 s at 66 °C, and 1 min at 72 °C. A final extension step was performed for 5 min at 72 °C. The amplification products with 500 bp in length were separated by electrophoresis on 1.0% agarose gels treated with GoldView II staining.

2.4. Gene expression analysis by qRT-PCR

Total RNAs were isolated from the transgenic lines and NT control using RNAsimple Total RNA Kit (lot#N2822, TIANGEN, Beijing, China) following the manufacturer's instructions. Reverse transcription was performed in 20 μL reaction mixture with the RevertAid First Strand cDNA Synthesis Kit (Cat No: 3K1622, Thermo Scientific) and qRT-PCR amplification was performed in 20 μL of the reaction mixture with the SuperReal PreMix Plus (SYBR Green) (lot#N3113, TIANGEN, Beijing, China), 10 μM of each primer (*ef1a* as an internal control gene and forward and reverse primers: 5′-CAAGGATGACCCAGCCAAG-3′ and 5′-TTCCTT ACCTGAACGCCTGT-3′, and the gene-specific forward and reverse primers: 5′-CTCACCACTTACGCACAAGC-3′ and 5′-CGGCAATATCCTCTTTCTCG-3′). Reactions were conducted with an ABI3000 device (Applied Biosystems 3000 Real-Time PCR System) using the default cycling conditions (30 s at 95 °C and 40 cycles of 95 °C for 5 s, 60 °C for 34 s, 15 s at 95 °C, 1 min at 60 °C and 95 °C for 15 s). Each experiment was repeated three times independently. After