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Proteomic analysis of canola root inoculated with bacteria under salt stress

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ABSTRACT

Plant-growth promoting bacteria can ameliorate the negative effects of salt stress on canola. To better understand the role of bacteria in canola under salt stress, salt-sensitive (Sarigol) and salt-tolerant (Hyola308) cultivars were inoculated with *Pseudomonas fluorescens* and protein profiles of roots were compared. Bacterial inoculation increased the dry weight and length of canola roots under salt stress. Using a gel-free proteomic technique, 55 commonly changed proteins were identified in Sarigol and Hyola308 roots inoculated with bacteria under salt stress. In both canola cultivars, proteins related to amino acid metabolism and tricarboxylic acid cycle were affected. Hierarchical cluster analysis divided the identified proteins into three clusters. Proteins related to Clusters II and III, which were secretion-associated RAS super family 1, dynamin-like protein, and histone, were increased in roots of both Sarigol and Hyola308 inoculated with bacteria under salt stress. Based on pathway mapping, proteins related to amino acid metabolism and the tricarboxylic acid cycle significantly changed in canola cultivars inoculated with or without bacteria under salt stress. These results suggest that bacterial inoculation of canola roots increases tolerance to salt stress by proteins related to energy metabolism and cell division.

Biological significance

Plant-growth promoting bacteria as an emerging aid can ameliorate the negative effect of salt stress on canola. To understand the role of bacteria in canola under salt stress, salt sensitive Sarigol and tolerant Hyola308 cultivars were used. Dry weight and length of canola root were improved by inoculation of bacteria under salt stress. Using gel-free proteomic technique, 55 commonly changed proteins identified in Sarigol and Hyola308 inoculated with bacteria under salt stress. In both canola cultivars, the number of proteins related to amino acid metabolism and tricarboxylic acid cycle was more than other categories with higher change in protein abundance. Hierarchical cluster analysis divided into 3 clusters. Cluster II including secretion-associated RAS super family 1 and dynamin-like protein and Cluster III including histones H2A were increased by bacterial inoculation in both cultivars.

Abbreviations: LC, liquid chromatography; MS, mass spectrometry; P5CS, delta 1-pyrroline-5-carboxylate synthase.

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Furthermore, pathway mapping highlighted the importance of S-denosylmethionine synthetase and malate dehydrogenase that decreased in canola inoculated with bacteria under salt stress. These results suggest that bacterial inoculation helps the canola to endure salt stress by modulating the proteins related to energy metabolism and cell division.

1. Introduction

Due to the apparent health benefits of reducing saturated fat intake in the human diet, canola is being increasingly used as a source of edible vegetable oil [1] and represents a third of all vegetable oil produced worldwide [2]. Because of the limited agricultural land, increasing plant productivity is necessary to generate sufficient plant oil for use in food and animal feed [3]. Although maximum yields of canola are obtained under normal soil and environmental conditions, the quantity and quality of seed yields are affected by environmental stress [4,5]. Improving the tolerance of canola to stressful conditions would lead to increased yields of higher quality oil.

Soil salinity is a major agricultural concern as it causes substantially or partially unproductive lands [6]. High salt concentrations of soil are often associated with ion imbalances and hyperosmotic pressure, which eventually lead to oxidative stress conditions for plants [7]. Tolerance against salinity stress is a complex trait that is governed by numerous mechanisms at the cellular, tissue, organ, and plant level [8]. Canola is categorized as a moderately salt-tolerant plant, with amphidiploid species being relatively salt tolerant in comparison with diploid species [9]. In particular, the amphidiploid species *Brassica napus* was found to have superior resistance among canola species in early growth stages in a comparative study of salt tolerance [10]. To improve the tolerance and increase the yields of canola exposed to salt stress, it is necessary to determine the underlying salt responsive and tolerance mechanisms.

Plant roots serve as a niche for the proliferation of certain species of soil bacteria. These plant–microbe interactions can be beneficial, neutral, or deleterious for plant growth [11]. For example, bacteria can improve plant growth and vigor in stressful environments by the fixation of nutrients [12], reducing the toxicity of heavy metals [13], controlling of soil-borne pathogens [14], and altering plant hormonal balance [15]. One well-characterized plant growth-promoting bacterium is the *Pseudomonas fluorescens* FY32, which increases the growth and yield of crops, particularly root length under salt stress [15,16]. Plant-growth promoting bacteria have the potential to aid crop production by reducing the deleterious effects of salt stress.

Soil bacteria moderate the harmful effects of salinity on canola. Proteomic analysis of canola roots revealed that many proteins involved in photosynthesis, anti-oxidative processes, transportation across membranes, and pathogenesis-related responses were differentially expressed in the presence of bacteria and salt [17]. In experiments with salt-tolerant (Hyola308) and salt-sensitive (Sarigol) cultivars, Bandehagh et al. [18] demonstrated that the levels of proteins related to oxidative stress and energy production were changed in response to salt stress. Using a similar approach, plasma-membrane, stress, and protein synthesis-associated proteins were found to play an important role in resistance against drought stress [19]. To better understand the stress-responsive mechanisms of canola, stress-tolerant and -sensitive cultivars are therefore useful materials.

To increase the quantity and quality of canola seed yields, it is necessary to develop cultivars with higher stress tolerance. In this study, to better understand the salt-responsive mechanisms of canola and plant growth-promoting bacteria in moderating the harmful effects of salt stress, salt-tolerant Hyola308 and salt-sensitive Sarigol canola cultivars were used. A proteomic technique was used to identify responsive proteins in canola inoculated with the plant-growth promoting bacterium *P. fluorescens* FY32 [20] under salt stress. In addition, to determine the role of the key proteins involved in the canola response to salt stress and bacterial inoculation, hierarchical cluster and pathway analyses were performed.

2. Materials and methods

2.1. Plant material

Canola seeds (*B. napus* L. cultivars Hyola308, Sarigol, RGS003, Amica, Hyola420, and Olga) were obtained from the Seed and Plant Improvement Institute (Karaj, Iran). Seeds were sterilized [21], germinated under aseptic conditions, transplanted, and cultured in a hydroponic system with sterilized Hoagland's solution [22]. The greenhouse was controlled as follows: temperature (25 ± 2 °C during the day and night), relative humidity (50% during the day and 60% at nights), light (14 h daily), and nutrient solution (pH 6.5 ± 0.5 using hydrochloric acid/potassium hydroxide).

2.2. Preparation of bacteria suspension

The bacterial strain *P. fluorescens* FY32 was aerobically grown in 250 mL Luria-Bertani medium [23] at 30 °C for 18 h with continuous shaking. The bacterial cells were harvested by centrifugation at 8000 ×*g* for 10 min at 4 °C and re-suspended in 25 mL sterilized 0.03 M $MgSO_4$ on ice [20]. To determine the population count of cultured bacteria, McFarland's method [24] was used. The absorbance of cell suspension was measured at 600 nm and the turbidity of bacterial suspension was adjusted to a concentration of approximately 10^{10} cfu mL^{-1} using sterilized 0.03 M $MgSO_4$.

2.3. Salt treatment and bacterial inoculation

One-week-old canola plants were transferred to a hydroponic system, which was sterilized and washed twice prior to transplantation. After transplantation, 10 mL of bacterial suspension in 0.03 M $MgSO_4$ was injected into each reservoir containing 10 L of nutrient solution. One week after inoculation, plants were treated without or with 150 and 300 mM NaCl.