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Review

Protein hydrolysates as biostimulants in horticulture

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A B S T R A C T

In recent years, new strategies have been proposed in order to improve the sustainability of production systems for horticultural crops. A promising tool would be the use of substances and/or microorganisms defined also as 'biostimulants' able to enhance crop quality parameters, nutrient efficiency and abiotic stress tolerance. Protein hydrolysates (PHs) are an important group of plant biostimulants based on a mixture of peptides and amino acids that have received increasing attention in the recent years due to their positive effects on crop performances. PHs are mainly produced by enzymatic and/or chemical hydrolysis of proteins from animal- or plant-derived raw materials. The current review gives an overview of the biostimulant properties of PHs on productivity and product quality of horticultural crops, in particular fruit trees, vegetables, flower crops and ornamentals. After a brief introduction on PHs as plant biostimulants, this review focuses on the classification and chemical composition of PHs according to the source of proteins and method of protein hydrolysis. The plant uptake and transport of amino acids and peptides and the effects of PHs on primary and secondary metabolism as well as the biochemical and physiological processes conferring tolerance to abiotic stress are also covered. The review concludes by proposing several perspectives for future research aiming to understand the mode of action of PHs based on their composition and also to define the suitable time and dose of application.

Contents

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1. Introduction

The world's horticultural systems face a great balancing act between two needs: (1) raise the supply of food produced on the available farmland, since the global population will increase to more than 9.3 billion by 2050, and (2) reduce agriculture's impact on the environment and human health (Searchinger, 2013). Meeting these two targets presents a major sustainability challenge to scientists and producers, which might be fostered by using natural products known as plant biostimulants (Calvo et al., 2014). Protein hydrolysates (PHs) are a category of plant biostimulants defined as 'mixtures of polypeptides, oligopeptides and amino acids that are manufactured from protein sources using partial hydrolysis' (Schaafsma, 2009). There has been growing interest in PHs during recent years due to their positive effects on crop performances, especially under environmental stress conditions (du Jardin, 2012).

PHs are mainly produced by chemical (with strong acids or alkalis) and/or enzymatic hydrolysis of proteins contained in agro-industrial by-products from animal (i.e., leather, viscera, feathers, blood) or plant origin (i.e., vegetable by-products) and in biomass of dedicated legume crops (i.e., seeds, hay) (Maini, 2006; Schiavon et al., 2008; du Jardin, 2012; Halpern et al., 2015 and references cited therein). PHs obtained by agro-industrial by-products represent a sustainable solution to the problem of waste disposal, making their production interesting from environmental and economical points of view (Kasparkova et al., 2009; Pecha et al., 2012; Baglieri et al., 2014). Actually, more than 90% of the PH market in horticulture is based on products obtained through chemical hydrolysis of proteins from animal origin (e.g., collagen from leather by-products in Europe, India and China; fish by-products in United States), while the enzymatically produced PHs from plant biomass are less common because they have been recently introduced to the biostimulant market.

PHs have been identified to improve the performance of several horticultural crops, including increased shoot, and root biomass and productivity (Kunicki et al., 2010; Lisiecka et al., 2011; Paradikovic et al., 2011; Colla et al., 2014; Ertani et al., 2014). Application of PHs to plant leaves and roots has been shown to increase Fe and N metabolism, nutrient uptake, and water and nutrient use efficiencies for both macro and microelements (Cerdán et al., 2009; Ertani et al., 2009; Halpern et al., 2015). The higher nutrient uptake in PH-treated plants has been attributed to (1) an increase in soil microbial activity and soil enzymatic activities, (2) improvement of micronutrient mobility and solubility, in particular Fe, Zn, Mn and Cu, (3) modifications in the root architecture of plants, in particular root length, density and number of lateral roots and, (4) an increase in nitrate reductase, glutamine synthetase and Fe(III)-chelate reductase activities (Cerdán et al., 2009; Ertani et al., 2009; García-Martínez et al., 2010; Colla et al., 2014; Lucini et al., 2015). PHs could also interfere with the phytohormone balance of the plant, thereby influencing plant development due to the presence of specific peptides and precursors of phytohormone biosynthesis, such as tryptophan (Colla et al., 2014). Several bioactive peptides produced in a variety of plants have been identified to have hormone-like activities (Ito et al., 2006; Kondo et al., 2006). Moreover, many scientific papers reported that the application of plant-derived PHs elicited auxin- and gibberellin-like activities and thus promoted crop performances (Schiavon et al., 2008; Ertani et al., 2009; Matsumiya and Kubo, 2011; Colla et al., 2014). PHs have been shown not only to improve plant nutrition but also the quality of fruits and vegetables in terms of phytochemicals (i.e., carotenoids, flavonoids, polyphenols) (Parrado et al., 2007; Paradikovic et al., 2011; Gurav and Jadhav, 2013; Ertani et al., 2014), and they can reduce undesired compounds, such as nitrates (Liu et al., 2008). In addition, PH application has been also been shown to avoid or reduce losses in production caused by unfavourable soil conditions and environmental stresses. These include thermal stress, salinity, drought, alkalinity, and nutrient deficiency (Botta, 2013; Cerdán et al., 2013; Ertani et al., 2013; Colla et al., 2014; Petrozza et al., 2014; Lucini et al., 2015; Visconti et al., 2015). However, the maximum benefits from PHs are obtained at very low dosages (Ertani et al., 2014) and are dependent on species/cultivar, environmental conditions, phenological stages, time and mode of applications (foliar vs. root) and leaf permeability to the biostimulant (Kauffman et al., 2007; Kunicki et al., 2010; Ertani et al., 2014). The later factor is crucial, since biostimulants are usually foliarly applied; hence, their penetration into plant tissue is a necessary condition for a reliable efficiency (Pecha et al., 2012).

Phytotoxic effects and growth depression of fruiting crops have been also reported after repeated applications of animal-derived PH products (Cerdán et al., 2009; Lisiecka et al., 2011), whereas no phytotoxicity and growth reduction was recorded in tomato plants (*Solanum lycopersicum* L.) after applications of a plant-derived PH (Cerdán et al., 2009). Similarly, several trials carried out at the University of Tuscia-Italy on basil demonstrated that foliar spray of some commercial animal-derived PHs caused leaf chlorosis, whereas no injury symptoms were recorded in basil after foliar spraying with the commercial plant-derived PH 'Trainer', up to 10 times the recommended dose. The detrimental effect of some animal derived-PHs on plant growth can be attributed to an unbalanced amino acid composition (Oaks et al., 1977), higher concentration of free amino acids (Moe, 2013) and high salinity (Colla et al., 2014). Besides phytotoxicity effects, there is an increased concern on the use of animal-derived PHs in terms of food safety, as demonstrated by the European Regulation No. 354/2014, which prohibited the application of these products on the edible parts of organic crops. However, Corte et al. (2014), evaluating safety and fertiliser efficacy of animal-derived PHs, concluded that PHs did not negatively affect eukaryotic cells and soil ecosystems, and PHs can be used in conventional and organic farming without posing harm to human health and the environment.

In this review, we focussed on recent advances in the biostimulant properties of PHs on growth, yield and product quality of horticultural crops (fruit trees, vegetables, flower and ornamental crops). The effects of PH on the primary and secondary plant metabolism and physiology as well as the tolerance to unfavourable chemical soil conditions and environmental stresses are covered.

2. Classification and chemical characteristics

PHs can be classified on the basis of protein source, and method of protein hydrolysis (Fig. 1). Production process and protein source strongly affect the chemical characteristics of PHs. Chemical hydrolysis of proteins under acid or alkaline conditions is usually preferred for producing animal-based PHs. Acid hydrolysis is a very aggressive process carried out at high temperature (>121 °C) and pressure (>220.6 kPa). In acid hydrolysis, hydrochloric and sulphuric acid are mainly used to hydrolyse proteins, the most common being hydrochloric acid (Pasupuleti and Braun, 2010). Alkaline hydrolysis is a fairly simple and straightforward process where proteins are solubilised by heating followed by the addition of alkaline agents, such as Ca, Na or potassium hydroxide, and maintaining the temperature to a desired set point (Pasupuleti and Braun, 2010). Chemical hydrolysis attacks all peptide bonds of proteins, leading to a high degree of protein hydrolysis (high content of free amino acids in total) and destruction of several amino acids (e.g., tryptophan is usually totally destroyed with acid hydrolysis; cysteine, serine and threonine are partially lost; and asparagine and glutamine are converted to their acidic forms with acid hydrolysis). Moreover, other useful thermolabile compounds (e.g., vitamins) are also mostly destroyed during chemical hydrolysis. One other crit-