I biostimolanti nei sistemi colturali ortofloricoli a basso impatto ambientale

Riassunto. I biostimolanti rappresentano dei mezzi tecnici di supporto alla produzione dei sistemi colturali, con la capacità di aumentare la potenzialità produttiva delle colture e la tolleranza agli stress abiotici. L’agricoltura moderna è sempre più attenta alla riduzione dell’impatto ambientale per cui tutti i sistemi agricoli stanno evolvendo verso metodi di coltivazione maggiormente eco-sostenibili, sia tradizionali sia biologici. L’obiettivo delle nuove pratiche agronomiche è quello di ridurre gli input senza diminuire le produzioni e la loro qualità. I biostimolanti sono composti da una o più sostanze bioattive, in gran parte ancora sconosciute, che esplicano un’azione generalmente positiva nei confronti delle colture, aumentando l’efficienza d’uso degli elementi nutritivi e la tolleranza agli stress abiotici. Nelle colture orticole, l’uso di biostimolanti permette di ridurre l’apporto di fertilizzanti senza compromettere la resa e la qualità del prodotto. Negli ortaggi da foglia sensibili all’accumulo di nitrati, come la rucola, i biostimolanti hanno la capacità di incrementare la qualità e mantenere il livello di nitrati sotto i limiti di legge. Nelle colture floricole, i biostimolanti sono normalmente utilizzati per aumentare la crescita e stimolare la fioritura.

Parole chiave: floricoltura, nutrizione, orticoltura, qualità, resa, stress.

Introduction

Biostimulants are products derived from organic material containing bioactive substances and/or microorganisms able to improve crops performance. These products, applied directly to the plants or to the soil (rhizosphere), stimulate several physiological and molecular processes that lead to increased/improved uptake and nutrients use efficiency, enhanced tolerance to abiotic stresses and produce yield and quality (The European Biostimulant Industry Council, EBIC, Du Jardin 2015; Yakhin et al., 20017; Rouphael and Colla, 2018).

The first biostimulants were referred to algae extracts that once applied as manure, already in Roman time, improved the soil fertility and the plant growth (Craigie, 2011). Over time, biostimulant products have increased and nowadays the category includes different kind of substances obtained from several raw materials.

In Italy, biostimulant have been included in the Annex 6 of legislative decree 75/2010 as “Products with specific action on plant”. Biostimulants are defined as materials that added to another fertilizer or to the soil or plant, could favor or regulate the absorption of nutrients or correct certain physiological disorders.

According to the classification given in Annex 6, biostimulant activities can be ascribed only to the following products:

- Alfalfa protein hydrolysate
- Animal hydrolyzed epithelium
- Alfalfa, algae and molasses liquid extract
- Alfalfa, algae and molasses solid extract
Toscano et al.

- Extract acid Algae fucales
- Mycorrhizal fungi inoculum
- Fabaceae enzymatic hydrolysate

In June 2011, the European Biostimulant Industry Council (EBIC) was established and a precise definition of biostimulants was given. A classification has been proposed and a wide collection of studies have been evaluated in order to reach an European regulation. At EU level, biostimulants are defined as substances or materials (not including nutrients and pesticides) which when applied to the plant, seeds or growth substrate in specific formulations can modify the physiological processes of plants by improving growth, development and/or increase the tolerance to abiotic stresses (Du Jardin, 2015).

According to Du Jardin, biostimulants can be classified as follows:
- Humic substances
- Complex organic materials
- Beneficial chemical elements
- Inorganic salts included phosphorus
- Seaweed extracts
- Chitin and derivatives of chitosan
- Antitranspirants
- Amino acids and other nitrogenated compounds

In 2013, EBIC elaborates a further definition of biostimulant: “Biostimulants are substances and/or microorganisms that applied to the plant or rhizosphere stimulate natural processes that improve the efficiency of absorption and assimilation of nutrients, abiotic stress tolerance and product quality. Biostimulants have no effect on parasites and pathogens and therefore do not fall under the category of pesticides”.

In 2015, the European Commission published a Road map for the new regulation. A biostimulant product may be marketed in all member states if it complies with the European Regulation, instead if registered in accordance with the national legislation, be marketed only in the member states where it has been registered as a biostimulant.

Currently the Italian legislation recognizes three types of products according to their action:
- Products that act as fertilizer adjuvants (inhibitors, coating, co-formulants and activators)
- Products that act on soil (fungal inocula)
- Products that act on plant (biostimulants)

Most commercially used biostimulants in the world are composed by:
- Humic substances
- Hydrolysed proteins and amino acids
- Microorganisms
- Seaweed extracts

In March 2016, the European Commission prepared a draft for a new fertilizer regulation. This proposal set out rules about the conversion of bio-waste into raw materials that can be transformed into fertilizing products. Moreover, it underlines that biostimulant category must not include products with a direct effect on biotic stress. In this case the product should be registered as plant protection product. The European Parliament approved the new EU Fertilising Products Regulation on 24 October 2017.

In November 2018, the final text of the EU fertilizing regulation appears almost ready that should be implemented by 2022.

**Biostimulants in the horticultural research**

The researches related to the biostimulants application for improving crop yield and quality have been increasing in the recent years. Most of the studies focus on the evaluation of biostimulant during crop cultivation or before and after an abiotic stress. The published papers have been dramatically increased in this area starting from the 2011 (fig. 1). The total number of scientific papers from 1961 to 2018 has been 543 among them 390 are research articles. The number of publication will surely increase.

Italy and United States are the countries that published the highest number of the papers. This high number of publications can be explained considering that in Italy and in US as well there are several fertilizers and biostimulants production companies. Most of them collaborate with the Universities and research centers.

The biostimulants have been widely applied in different crops and the efficacy has been variable because often the optimal concentrations and the application timing as well as the crop responses cannot be generalized all plant species. The research activities should focus on the understanding the mode of action of the biostimulants in the plants and identify the target physiological pathways to obtain information that can be exploited in different crop management systems.

The main research topics (fig. 2) for the biostimulant characterization are:

- **Identification of the biostimulant composition**: this issue is the most critical aspect of the biostimulants, because the raw materials used and the industrial process for the production can affect the final composition of biostimulants. These are a mixture of fermented or extracts organic materials, therefore, the exact composition of each single element is impossible to obtain. Usually, the
information, that can be easily reported, are represented by the mineral element concentrations, the amino acids content, the phenols concentrations, sugars, and plant hormones. Moreover, most of these compounds are very low in concentrations and are below the detection thresholds of the most innovative analytical instruments. In the future, surely, the analytical procedures will improve, and the information relative to the biostimulant composition will improve, but nevertheless the determination of the exact composition will remain a problem for these products.
• **Standardization of the production procedures:** the industrial biostimulant production process must be standardized in order to guarantee the same chemical and biological characteristics of the biostimulants. The first step of the standardization starts from the selection of the raw materials. Further studies are required for the identification of the best extraction protocols for the different raw materials. Therefore, sequential temperature treatments or pressure, organic solvent, etc. should be studied in relation to the functional effect of the extract in plants.

• **Plant responses characterization:** physiological, biochemical, and molecular studies should be carried out to understand the metabolic changes induced by the biostimulant on plants. Since the composition of biostimulants cannot be exactly determined, the classification of them can be obtain through the evaluation of their effect on the primary and secondary metabolism of the plants. This reverse approach can be useful for the selection and characterization of the biostimulants based on plant responses. The idea is to link the unknown biostimulant composition to specific biological effect on plants.

• **Identify common and reproducible effect of biostimulants in crops:** another critical aspect of the biostimulants is the lack of efficacy in different plant species or even in different cultivars of the same species. This can happen because the bioactive compounds can have an activation effect at different concentration in different plants or on the other way around plants have different sensitivity thresholds to the bioactive compounds of the biostimulant. The research studies should identify the range of active concentration for each biostimulant.

• **Timing and doses:** biostimulants are often used to counteract abiotic stresses. The efficacy of the biostimulants also depends from the timing of application before, during or after the stressful event. Since biostimulants have to activate specific defense mechanisms, it is important to identify the best application time. The optimal dose is also very important because within a certain range the crop can positively respond to biostimulants application. Therefore, it is important to define for each biostimulant the optimal application range, too high or low concentrations can nullify the biostimulant effect (Vernieri et al., 2005).

The research activities for biostimulants production and application require a tight collaboration among different stakeholders: industrial companies, farmers, raw materials providers and research institutions. The biostimulants production is not yet ruled at EU level, therefore the research activities can provide useful information for define the correct legislations to apply for biostimulant production and use.

### Biostimulants in ornamental plants production

Floriculture is one of the most remunerative activities in the agricultural sector. The high aesthetic quality of the final product and harvest timing are basic growers’ targets to support successful business and market competitiveness. Therefore, ornamental productions are generally thought requiring high inputs in terms of agrochemicals and energy consumption and in this way managed when more holistic approaches are not considered. In the above scenario, the impact of agricultural activity is high on the environment and public opinion about sustainable-perceived production chains. A new generation of organic and inorganic biostimulant products is arising for applications in the sector of ornamental production that offer multiple advantages worth exploring. Most of them are commercially available while others, under investigation, are showing interesting perspectives to maximize the sustainability of ornamental plant cultivation. Nonetheless, the use of biostimulant molecules for the cultivation of hedonistic agricultural products is simpler than for food crops where the effects of such molecules on edible organs must be thoroughly evaluated in terms of human health impact. The application of biostimulants involves many aspects of the ornamental production chain from plant, propagation, cultivation to post-harvest. The main targets are the improved input (water, fertilizers, energy, etc.) use efficiency, enhanced quality and limited/zero use of chemical hormones and pesticides. Plant biostimulants have been meaningfully classified by du Jardin (2015); their classification is complex and biostimulant substances can have multiple effects on ornamental plants since biostimulation is often exerted through non-specific pathways triggering several different signaling molecules, which in turn cause various plant physiological responses. In this review, the relationship between biostimulants and their effects on ornamental plants is analyzed as a function of the above reported growers’ targets.

Among various aspects of the plant response to biostimulators, improved photosynthesis activity and/or improved conversion of photoassimilates into plant structural tissues are highly valuable variables. Indeed, enhanced growth rate can bring about shorter growing cycles with higher energy use efficiency, for
example through lower fuel consumption for heating (e.g., under protected cultivation), higher nutrient and water use efficiency, and, in general, improved tolerance to biotic and abiotic stress. Hybiscus plants, treated with hydrolyzed substances obtained from green compost and fraction of urban solid wastes (i.e., FORSU), showed enhanced photosynthetic rate that turned into higher relative growth rate, biomass accumulation under optimal growing conditions (Massa et al., 2016). The same products induced positive effects on plant photosynthesis in Euphorbia x lomi (Fascella et al., 2015) likely due to the presence of humic and fulvic acids (HS and FA, respectively), relatively high Si content, and presence of various bioactive organic molecules (complex organic materials). Indeed, HA and FA can exert hormone-like stimulation with positive effects on photosynthesis and related biomass accumulation in ornamental plants (Canellas et al., 2015) due to their action on higher plants physiological mechanisms (Nardi et al., 2002). In a greenhouse experiment, foliar applications of HA increased photosynthetic rate, chlorophyll content, and shoot and root biomass of chrysanthemum (Fan et al., 2014). In cut gladiolus, HA-treated plants showed shorter flowering cycle than untreated plants in concomitance with higher flower production and bigger shoot and corms size (Baldotto and Baldotto, 2013). In a study with humic substances, on woody ornamental plant (Lantana camara), genetic analyses highlighted the relationship between the above substances and the activation of genes involved in plant flower and fruit development (Calvo et al., 2014).

Biostimulant products are then much appreciated for their positive effects on ornamental plant growth and aesthetic appearance. For example, HA applications increased flower diameter in chrysanthemum (Fan et al., 2014). Protein hydrolysates were evaluated by De Lucia and Vecchietti (2012) who found out that these substances shortened the crop cycle and increased the diameter of flower buds of lily. Foliar spray or root drench applications of seaweed extracts (Ecklonia maxima) resulted in improved growth during the vegetative and reproductive phase of marigolds (van Staden et al., 1994). However, much attention has been, up to now, also paid on the use of microorganisms for the biostimulation of ornamental plants. Rhizobacteria belonging to the genus Azospirillum induced faster growing cycles, plant tissue characteristics, increased flower number and plant growth in gladiolus, and petunia compared with untreated plants while similar results were obtained for chrysanthemum, pelargonium, jasmine and zinnia plants treated with Pseudomonas (Ruzzi and Aroca, 2015). A consortium of rhizobacteria and Trichoderma viridae showed positive effects in terms of produce yield and quality in jasmine plants (Ruzzi and Aroca, 2015). The application of chitosan, copolymer of N-acetyl-d-glucosamine and d-glucosamine, has also been found to anticipate flowering and promote plant growth of many flower plants such as begonia, freesia, garden lobelia, gladiolus, gloxinia, lisanthius, monkey flower, Persian violet, and wishbone flower (Pichyangkura and Chadchawan, 2015). Among inorganic biostimulants, Si is probably the most investigated element. Application of Si was found to improve commercial quality and yield of rose (Hwang et al., 2005; Savvas et al., 2007) and gerbera (Savvas et al., 2002; Kamenidou et al., 2010). Drenching applications of Si ameliorated the produce quality of ornamental sunflower by increasing flower stem height and thickness, as well as flower diameter (Kamenidou et al., 2008).

On the other hand, the use of biostimulants may represent a concrete alternative to reduce chemical products for the agamic propagation of ornamental plants in low-input and chemical-free productions. In plant propagation, humic substances extracted from vermicompost were proposed as an alternative to synthetic hormones (i.e., IBA) in croton and hibiscus cuttings that showed higher elongation rate compared with control plants (Baldotto et al., 2012). The application of similar substances extracted from manure enhanced plant propagation performance in terms of acclimatization and final quality of orchid (Baldotto et al., 2014). Earlier rooting of photinia cuttings were observed in plants treated with the rhizobacteria Azospirillum brasilenses (Larraburu et al., 2007). Seedling treatment and soil applications of chitosan induced enhanced seedling growth in begonia, garden lobelia, gloxinia, lisanthius, monkey flower, Persian violet, and wishbone flower (Ohta et al., 2004). Yet, chitosan showed positive effects for in vitro-propagated ornamental plants (Jamal Uddin et al., 2001; Nge et al., 2006). The application of Si via mist was found to increase the percentage of rooting and emergence of new leaf in rose cuttings (Gillman and Zlesak, 2000). Many commercial biostimulant products have also been tested for improving the rooting of ornamental cuttings through alternative-to-chemical products. For example, the application of Activewave® to camellia cuttings was more effective in the stimulation of root growth than gibberellic acid (Ferrante et al., 2012). The effects of some commercial products on agamic propagation of ornamental plants were recently reviewed by Bulgari et al. (2015).
The massive use of fertilizers in ornamental crops is one of the main pollution sources for this agricultural sector, therefore high agronomic nutrient use efficiency is desirable for sustainable productions. The use of biostimulant organisms for improving plant nutrition has been deeply investigated in many studies addressing the positive role of arbuscular mycorrhizae in nutrient uptake, especially of phosphorus. In a pot trial carried out with three ornamental bedding plants (petunia, impatiens and aster), mycorrhized plants allowed saving 70% phosphorus fertilizers (Gaur et al., 2000). Humic-like organic substances were also found to improve nutrient use efficiency of potted ornamental plants grown under sub-optimal nutrient availability in the root zone (Massa et al., 2018). Yet, the negative effects of heavy metal were alleviated by the presence of mycorrhiza fungi in flower plants (Liù et al., 2011; González-Chávez and Carrillo-González, 2013).

Indeed, several organic and inorganic biostimulant products have been tested on ornamental plants to overcome the pressure of biotic and abiotic stresses. Although ornamental plants are preferably grown under no climate and input limitation, adverse cultivation and post-harvest conditions (e.g., during transport) may cause significant losses of produce quality and yield. In a pot experiment with bedding plants, seaweed extract of Ascophyllum nodosum were revealing positive effects on plant growth and development of petunia, pansy and cosmos exposed to drought (Battacharyya et al., 2015). The same products were successfully used in the cultivation of amaranth to partially overcome the negative effects of salt stress (Aziz et al., 2011). Salinity effects may be also alleviated using arbuscular mycorrhizae that can acts as powerful biostimulators of ornamental plants. Better performance, in terms of produce quality, were for example observed for mycorrhized carnation grown under salinity, which showed higher nutrient/saline ion ratio compared with untreated plants (Navarro et al., 2012). Similar results were obtained in the cultivation of euonymus irrigated with reclaimed water (Gómez-Bellot et al., 2015). Some interesting results are also arising from the use of rhizobacteria that have been found to increase salt tolerance of ornamental species belonging to different botanical families (Sharp et al., 2011; Damodaran et al., 2014). On the other hand, rose plants growing under salt stress showed enhanced tolerance if treated with Si applied hydroponically (Savvas et al., 2007). Similar results were also observed in the case of carnation (Soundararajan et al., 2015). Although salinity is one of the major constraints in the cultivation of many ornamental plants, especially those devoted to cut flowers, the use of moderate saline water for irrigation is becoming an exigency in many cultivated areas. This is the case of the Mediterranean basin where many agricultural activities are regarding ornamental plants production. Therefore, each strategy boosting cultivation under salinity is worth exploring for achieving high water use efficiency and crop sustainability in low-input and closed-loop irrigation systems.

Plant pathogens are generally controlled by massive use of pesticides in the ornamental sector where the final product is not allocated to the food market and then less subjected to limitations. The use of biostimulants or biocontrol compounds can improve plant defenses against pathogens and their application for plant protection is more common than for other agricultural practices. The lethal effects of Phytophthora cinnamomic were alleviated in ornamental Proteaceae treated with phosphite (Shearer and Crane, 2012). Chitosan and derived products were also used for plant protection. Spraying solution containing chitosan was helpful to protect symptomatic plants of chrysanthemum and rose against the detrimental effect of various fungal pathogens (Wojdyła, 2004). Finally, many microorganisms, playing a role of biocontrol agents against plant pathogens (e.g., Trichoderma spp.), can act as biostimulators helpful for a sustainable production of ornamental plants.

Biostimulants in organic vegetables production

In the last years, the interest in the use of biostimulants in organic vegetable production is strongly increased also due to increase of these agriculture production systems. The growing demand for food, feed, fuel, fiber, and raw materials and the increasing resource depletion and ecosystem degradation impose the use of more sustainable cultivation methods (Colla et al., 2014). For these reasons, the conversion of conventional farming to organic farming systems has been more extensive. Organic farming is generally characterized by lower crop yield compared with conventional production systems mainly because of the limitation imposed on fertilization (no use of chemical fertilizers) and on plant defense (no use of pesticides) (de Ponti et al., 2012; Orsini et al., 2016).

In this frame, the use of biostimulants, that are able to increase crop tolerance against abiotic stresses or improve nutrient use efficiency, plant health, productivity and yielding at different growth stages, is particularly interesting (Bulgari et al., 2014). Organic farming can benefit from the use of these substances
that can enhance plant resilience to the nutrient limitation typical of this production system, therefore reducing the gap between organic and conventional yields (De Pascale et al., 2017).

The greatest difficulty, in terms of the correct regulatory framework for these substances, is related to their heterogeneity. This situation gives insecurity for operators, control authorities and bodies that certify and control the organic production, and strongly limits the spread of these substances.

The use of biostimulants could be very important in improving agricultural sustainability, as they may facilitate enhanced production with lower environmental impact (Ertani et al., 2015). They play a role that complements plant protection products and fertilizers, as they act on plant vigor without protecting against pests, and improve the efficiency of nutrient use without providing nutrients. This ability can lead to a reduction in the amount of pesticides and fertilizers used, consequently having indirect positive effects on the environment.

Although it is assumed that biostimulants may be particularly useful in organic farming, the indications in the literature are not very extensive. This is due, partially, to the fact that some substances are not registered for organic farming; for instance, organic practices prohibit the use of synthetic chemical products so that the elicitor compounds should occur in nature and should not be derived from genetically modified organisms (Garcia-Mier et al., 2013).

Furthermore, the aspects linked to this cultivation systems are only recently subject to scientific attention, at least as regards intensive systems, which are those of the cultivation of vegetables.

In vegetables, the application of biostimulants allowed a reduction in fertilizers without affecting yield and quality. Amanda et al. (2009) evaluated the use of biostimulant for reducing nitrate content and improving the commercial quality of baby leaf lettuce. Moreover, in leafy vegetables, biostimulants increased leaf pigments (chlorophyll and carotenoids) and plant growth by stimulating root growth and enhancing the antioxidant potential of plants.

In a study conducted by Tarantino et al. (2015), on the qualitative characteristics of the crop products, the plants under the organic fertilization system (without and with biostimulants) showed higher percentages of dry matter (cauliflower corymbs) and lower concentrations of nitrates (pepper fruit, fennel bulbs) than the plants under the conventional system. This is particularly positive, as nitrates and nitrites accumulation in plant tissues can constitute a danger for human health.

Organic production is aimed at sustainable plant production that includes a diverse and active soil microbial community. Thus, organic horticulture perse is a benefit for arbuscular mycorrhizal fungi (AMF), as reported in many papers (Gosling et al., 2006; Galvan et al., 2009; Kelly and Bateman, 2010). Moreover, specific investigations have confirmed the hypothesis that higher AMF propagule numbers and diversity occurred in organic farming (Rouphael et al., 2015).

As described by Olivares et al. (2015), in tomato, humate and plant growth promoting bacteria applied as substrate to seedling growth, and/or by spraying plant leaves, significantly increased production of tomato during the first year of conversion from conventional to organic farming. The application of humates isolated from vermicompost in combination with diazotrophic endophytic bacterial inoculation appears to be a powerful biotechnological tool for plant growth promotion in sustainable agriculture systems (Canellas et al., 2015).

Plants grown in organic farming are often exposed to nutrient deficiency resulting from low amounts of nutrients in the soil or to the poor solubility of nutrients in soil solution. Plant biostimulants can enhance the nutrient availability for plant uptake by increasing cation exchange capacity of soil (reduction of nutrient leaching especially in sandy soils), by supplying nitrogen to the crops, and/or by enhancing the solubility of nutrients in soil solution. The effectiveness of humic substances (HS) to improve salinity tolerance was also reported by Türkmen et al. (2004) and Paksoy et al. (2010) on tomato and okra, respectively.

The use of C Ts (compost of tea) is spreading in organic farming worldwide (Shaheen et al., 2013) because of benefits they provide as fertilizer, biostimulant or foliar spray against pathogens. In particular, the effects of an aerated water-extracted CT obtained from vegetable composts, applied as foliar spray on pepper plants, was evaluated for two years. In the first year, total production increased by 21.9% whereas, in the second year, it increased by 16.3%. In both years, physiological and nutritional status of pepper plants were increased, as resulted by leaf-SPAD assessed during crop cycle. Findings indicate the effectiveness of CT application in improving significantly yield performances of vegetable crops under greenhouse organic farming system (Zaccardelli, 2018).

In a study different biostimulants [fennel processing residues (FPR), brewer’s spent grain (BSG), lemon processing residues (LPR)] were analyzed on tomato; FPR appears promising candidates for enhancing plant productivity and fruit quality. FPR and BSG increased fruit mineral content and BSG-
FPR-LPR in combination enhanced titratable acidity. FPR-treated fruits had also 20% more vitamin C than control, and higher phenol content was obtained in those of BSG-LPR (Chehade et al., 2018).

Conclusion

The biostimulants can represent useful tools for improving the crop performance reducing the inputs and environmental impact. The abiotic stresses are responsible for the reduction of yield, ranging from 50-70% in many agricultural crops. The application of biostimulants can enhance the crop tolerance and reduce the yield losses. In vegetables and floriculture, the biostimulants can also improve nutrient use efficiency in protected cultivation and open field. The biostimulants can partly substitute the chemical inputs (fertilizers, pesticides, and plant growth regulators) and can increase the yield and quality of many crops under organic management.

Abstract

Biostimulants are products obtained from different raw materials that are used in cropping systems to produce in a more environment-friendly and sustainable way. New cultivation systems aim to reduce the inputs without compromising yield or quality of the final products. These aspects are important in conventional but mostly in organic farming, where the use of fertilizers is limited. Biostimulants have been used as plant stimulators of growth under both optimal and stressful conditions. These compounds can modulate crop physiology and biochemistry by improving the nutrient use efficiency and increasing the tolerance against abiotic stresses. In vegetables farms, biostimulants have been used for lowering the nitrate accumulation which afflicts leafy vegetables market. In stressful environments, biostimulants can activate the secondary metabolism, such as phenylpropanoid pathways, and enhance the concentration of several bioactive compounds with potential benefit also for human health. In floriculture, biostimulants have been applied to speed up the growing cycles and enhance the flower production and nutrient use efficiency.

Keywords: ornamental plants, nutrition, vegetable crops, quality, yield, stress.

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