IL PRIMO ANNO DEL PROGETTO BIOSTIMOLA. PRESENTAZIONE DEI RISULTATI E DELLE ATTIVITÀ SVOLTE

La realtà dei biostimolanti a portata di campo

Workshop progetto BIOSTIMOLA

PRODOTTI AD AZIONE BIOSTIMOLANTE: ASPETTI FISIOLOGICI E QUALITÀ DELLE PRODUZIONI VEGETALI

ANDREA ERTANI
DISAFA, UNITO

Fondo Europeo Agricolo per lo Sviluppo Rurale: l'Europa investe nelle zone rurali

Iniziativa realizzata nell'ambito del progetto **BIOSTIMOLA**, cofinanziato dall'operazione 1.2.01 "Progetti dimostrativi e azioni di informazione" del Programma di Sviluppo Rurale 2014 – 2020 della Regione Lombardia.
Responsabile del progetto è il **DiSAA dell'Università degli Studi di Milano**, realizzato con la collaborazione di **Agricola 2000**

Biostimulants are not governed by any uniform classification anywhere in the world.

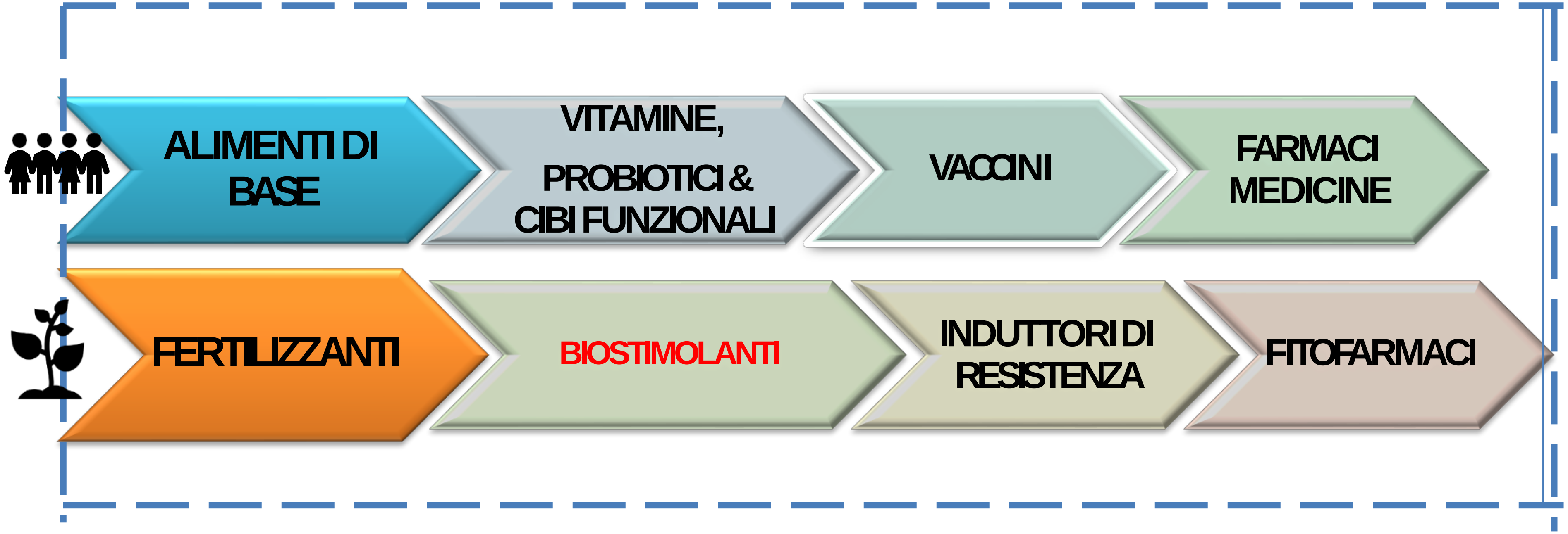
They are not classed as a distinct category with a standardized global definition.

According to EBIC, there is disagreement in Europe over whether biostimulants should be classified as **plant protection or plant nourishment**, resulting in a divided set of contradictory perspectives on the goods.

In France, they are classified as **additives**, whereas in Germany, they are sold as **plant straighteners or growth promoters**.
The European Biostimulants Industry Consortium (EBIC) has prepared a regulation based on the views and ideas of manufacturers and research institutes and put it for EU approval in order to consolidate the regulatory framework for biostimulants in Europe. However, biostimulant compounds must be repeatedly registered to assure compliance with the rules of each European country. The data requirements and parameter range also differ by EU member country.

Tuttavia, i composti biostimolanti devono essere registrati ripetutamente per garantire il rispetto delle norme di ciascun paese europeo. Anche i requisiti relativi ai dati e la gamma di parametri variano a seconda del paese membro dell'UE.

I BIOSTIMOLANTI OCCUPANO UN POSTO UNICO E RECENTEMENTE RICONOSCIUTO NELLO SPETTRO DEGLI INPUT DELLE COLTURE



Classificazione

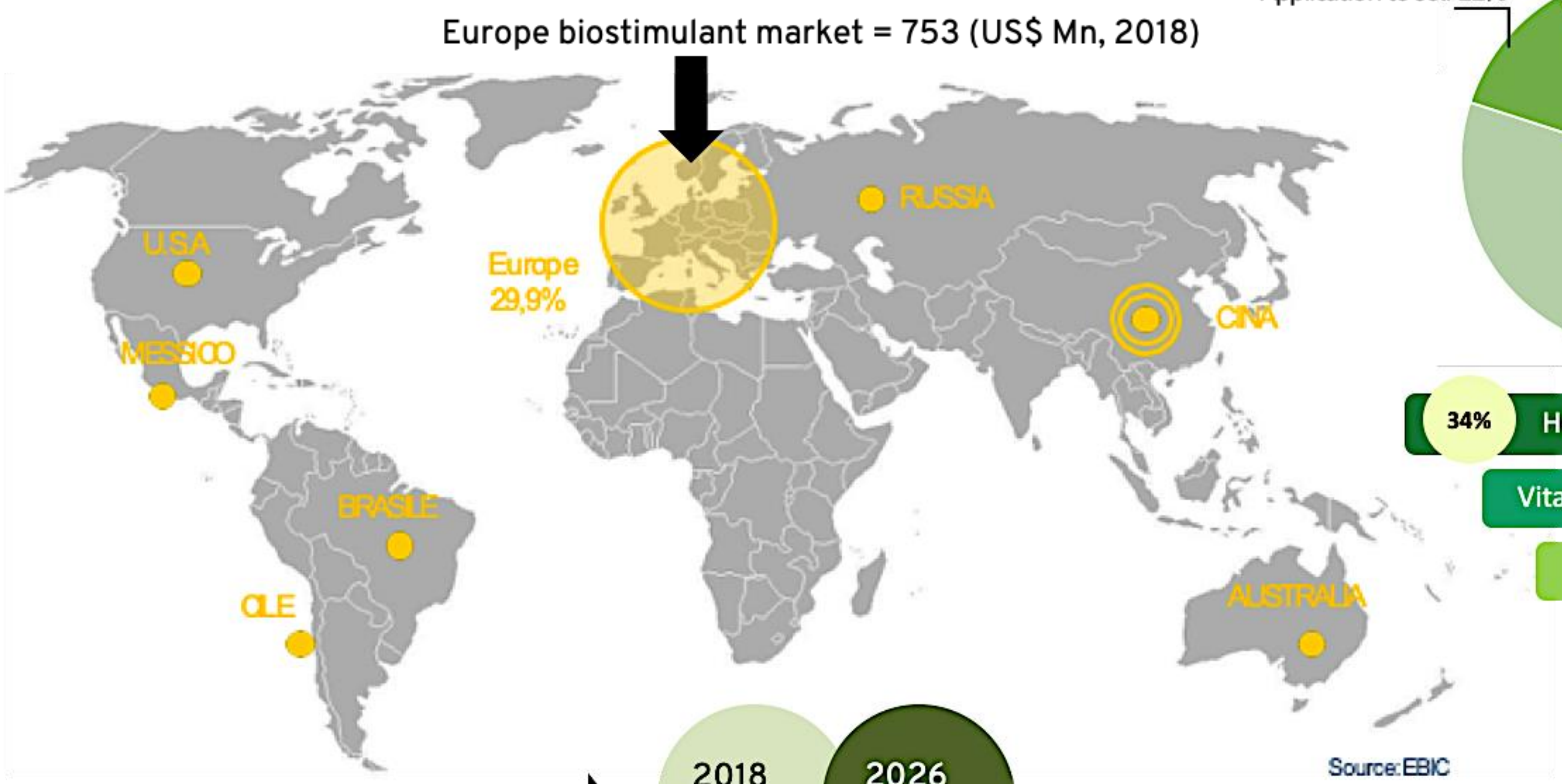
Biostimolanti microbici

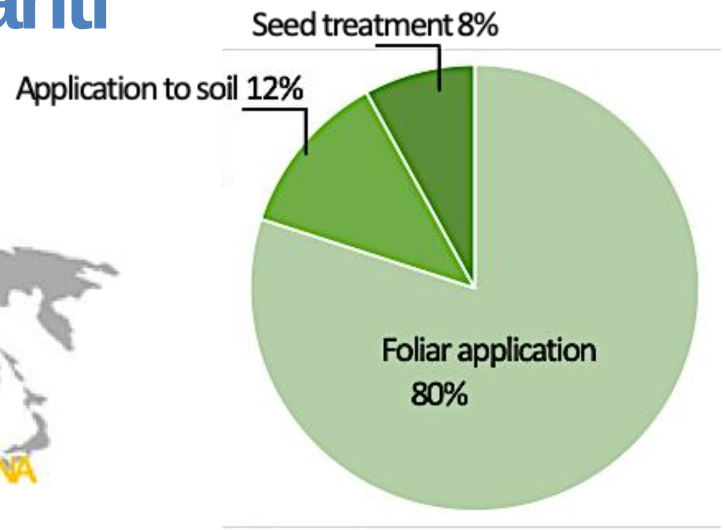
- Funghi micorrizici (VAM)
- Batteri promotori della crescita (PGPB)

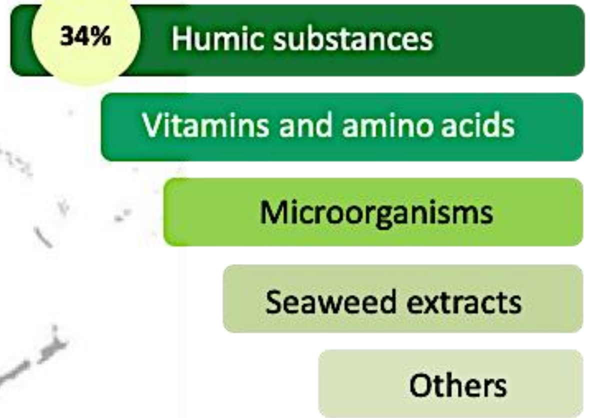
Biostimolanti non microbici

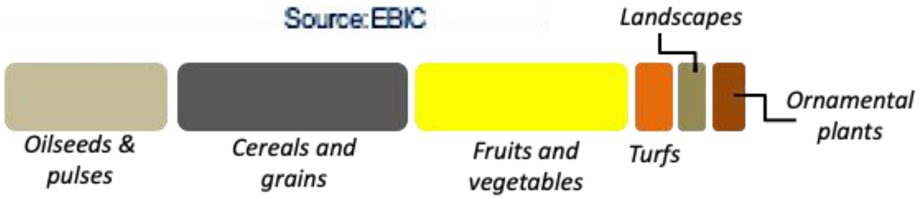
- Sostanze umiche
- Estratti di alghe (Ascophyllum, Fucus e Laminaria)
- Elementi benefici (e.g., silicio, selenio, cobalto, alluminio)
- Chitosano e altri biopolimeri
- Vitamine e aminoacidi
- Idrolizzati proteici (PHs)

BIOSTIMOLANTI gli 8 mercati più importanti









Risposte comuni indotte dai biostimolanti nelle piante: i meccanismi di induzione possono essere diversi e dipendono dalle caratteristiche chimiche e fisiche e dall'attività biologica delle singole formulazioni.

Contents lists available at ScienceDirect

Plant Science

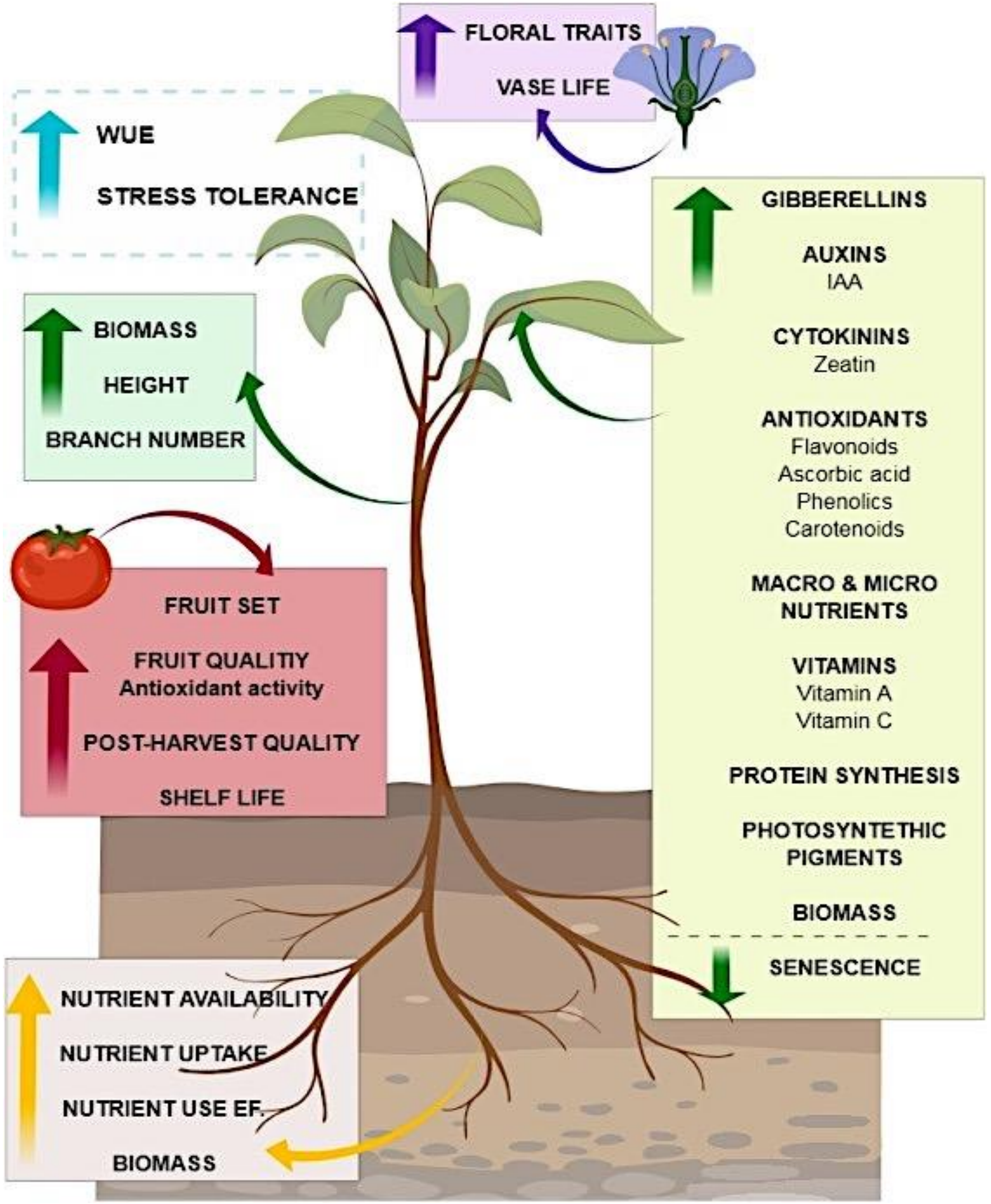
journal homepage: www.elsevier.com/locate/plantsci

Review article

An overview of plant-based natural biostimulants for sustainable horticulture with a particular focus on moringa leaf extracts

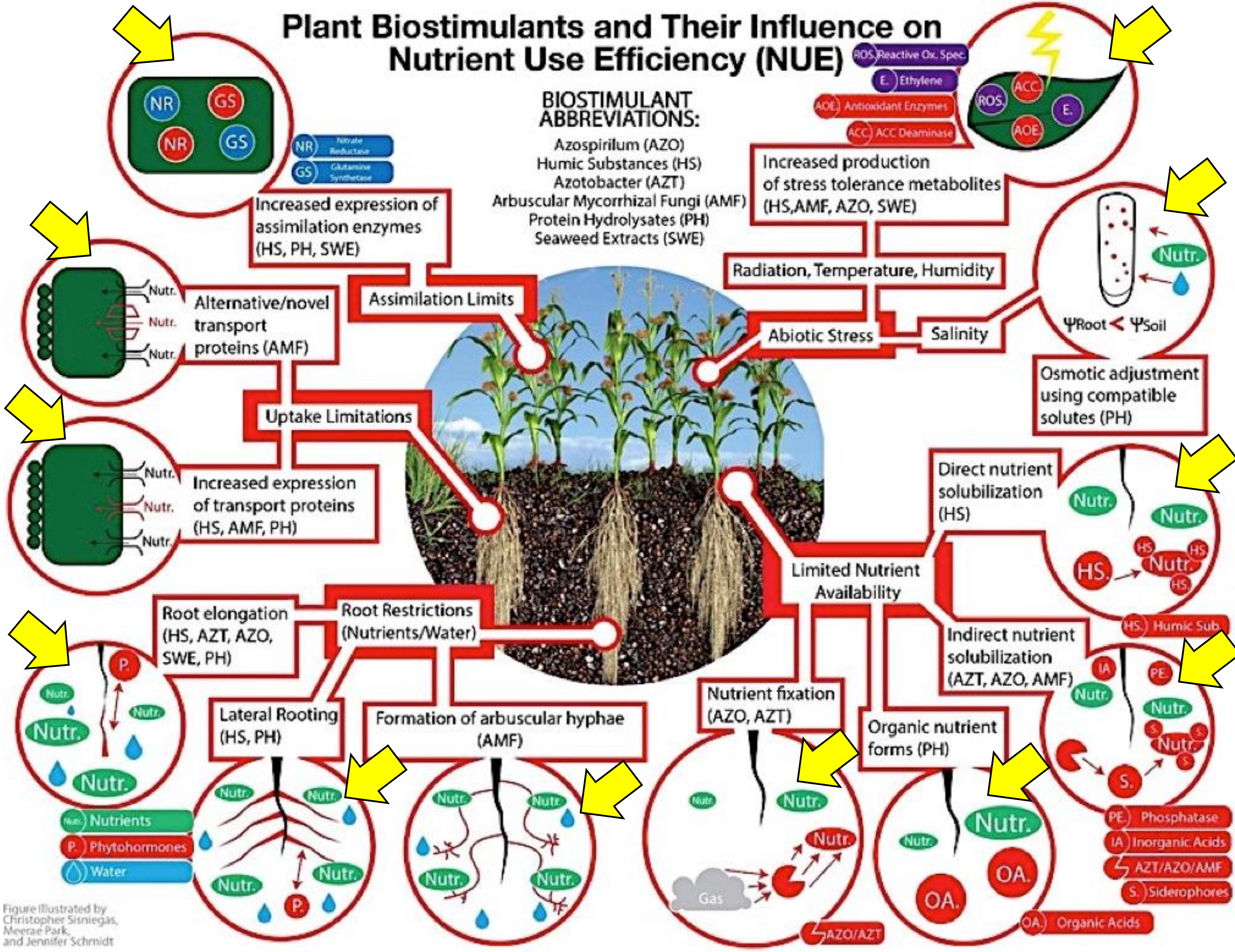
Faisal Zulfiqar[a], Andrea Casadesús[b], Henry Brockman[c], Sergi Munné-Bosch[b,d,*]

[a] Institute of Horticultural Sciences, Faculty of Agriculture, University of Agriculture Faisalabad, 38000, Pakistan
[b] Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, University of Barcelona, Avinguda Diagonal 643, 08028, Barcelona, Spain
[c] Department of Primary Industries and Regional Development Western Australia, 444 Albany Highway, Albany, 6330, Australia
[d] Research Institute on Nutrition and Food Security (INSA), University of Barcelona, Spain

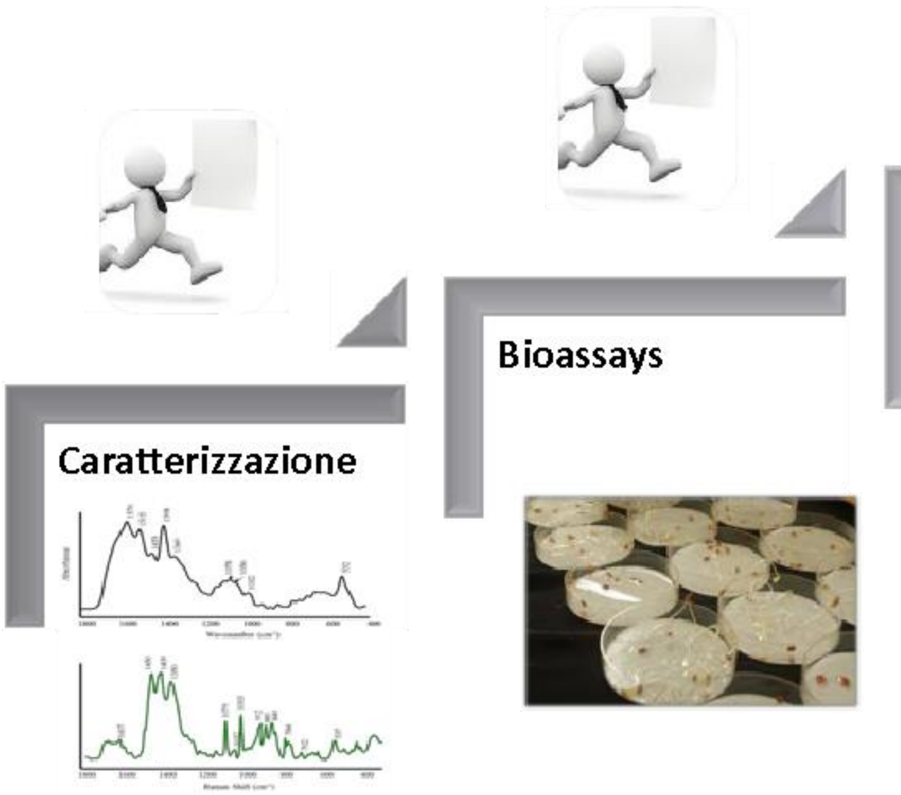


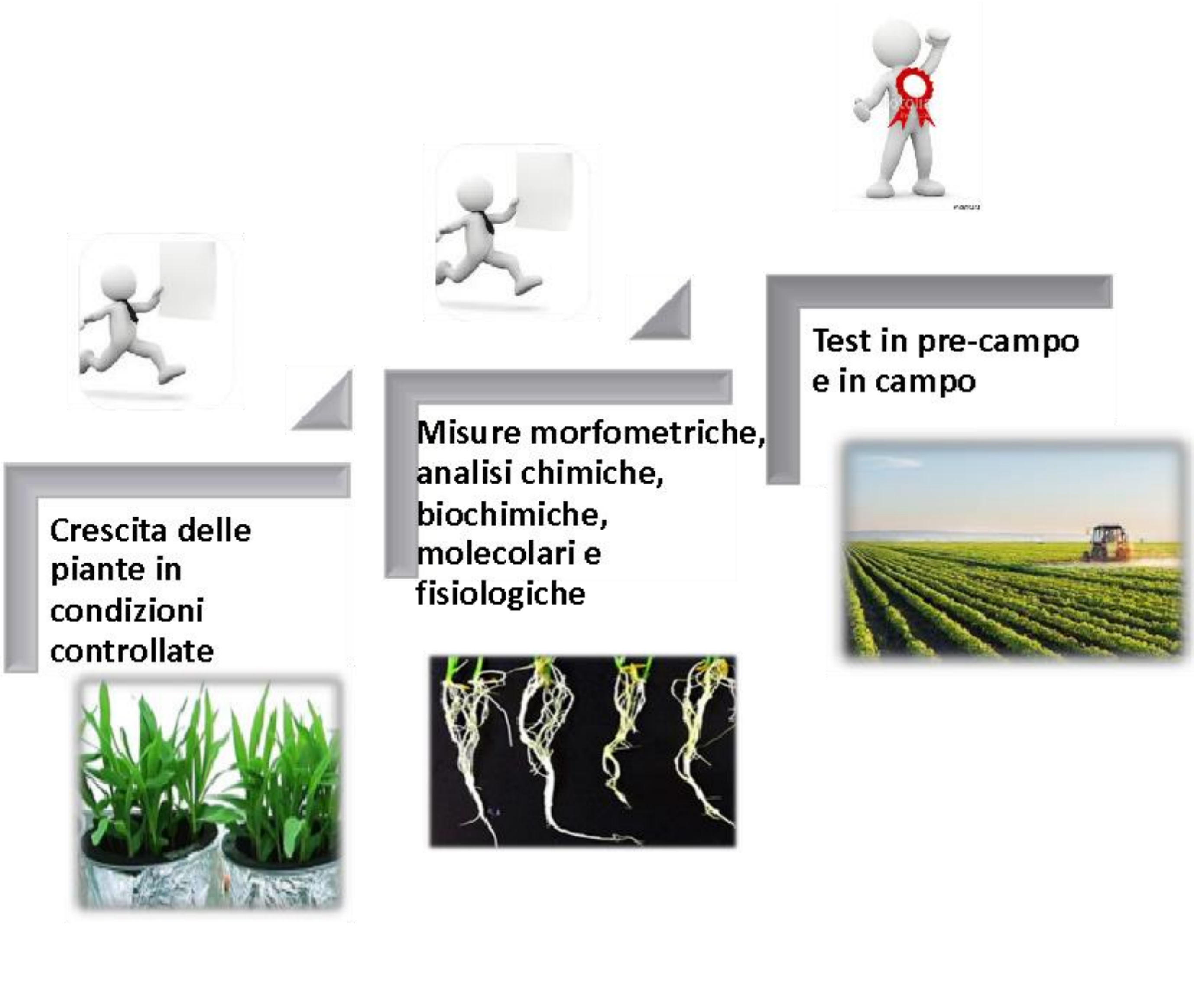
Effetti specifici indotti dalle diverse classi di biostimolanti nelle piante su:

- Assorbimento di nutrienti
- Assimilazione di nutrienti
- Mobilizzazione dei nutrienti nel suolo
- Resistenza a stress abiotici



La conferma dell’efficacia di un prodotto biostimolante e la comprensione del suo meccanismo d’azione richiede un iter complesso





Idrolizzati proteici (PH)

- ✓ Prodotti di origine diversa
- ✓ **Generati attraverso idrolisi termica, chimica o enzimatica**

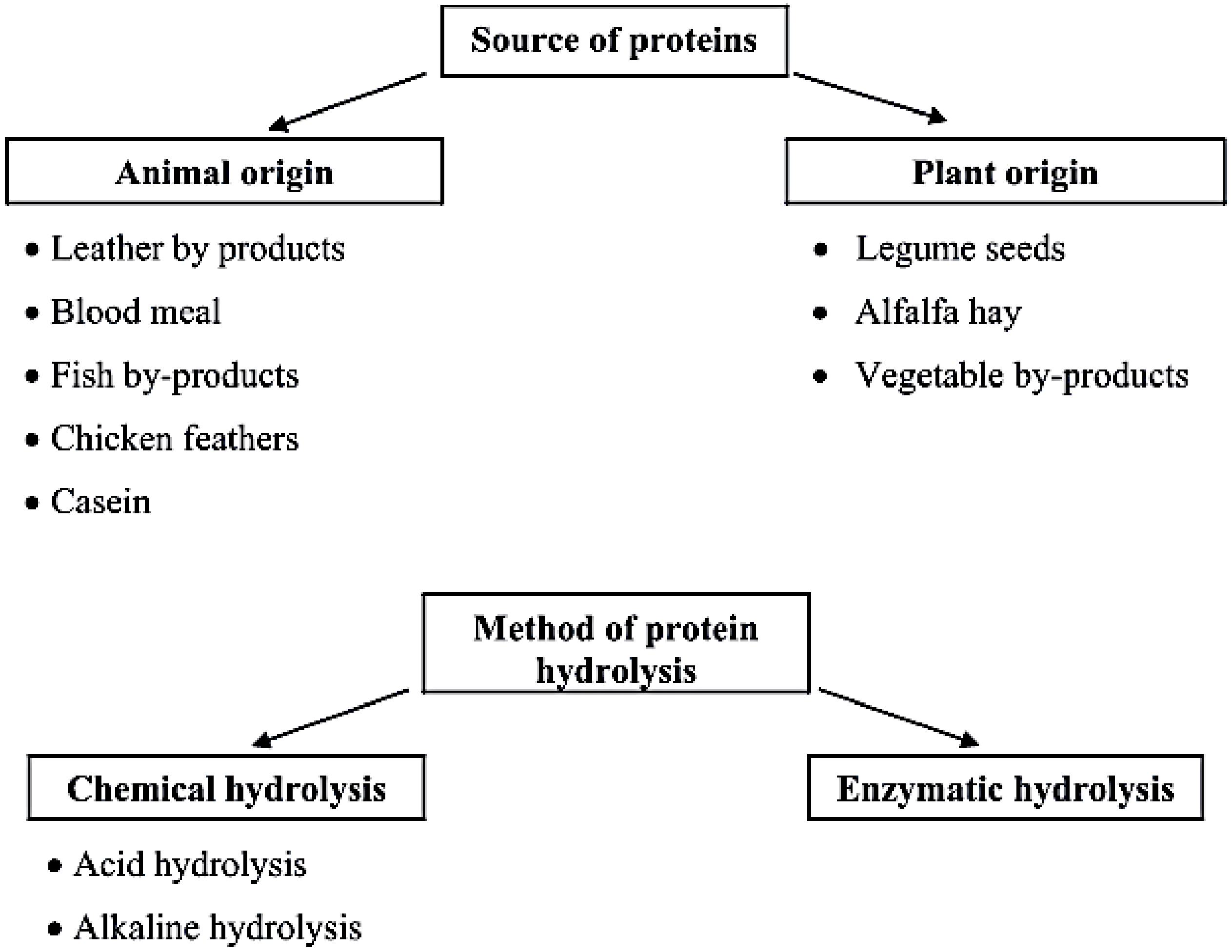


Fig. 1. Classification criteria of protein hydrolysates on the basis of protein source and the method of protein hydrolysis used in the production process.

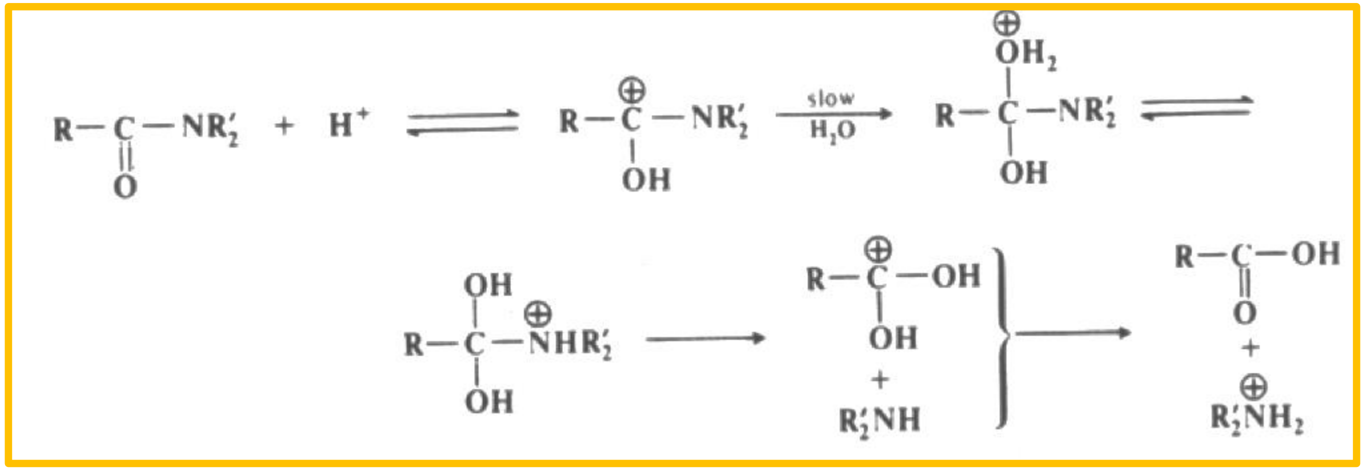
IDROLISI ED IDROLIZZATI PROTEICI

L'idrolisi è l'azione di scissione dei legami di un composto organico o inorganico in ambiente acquoso, con produzione di composti semplici.

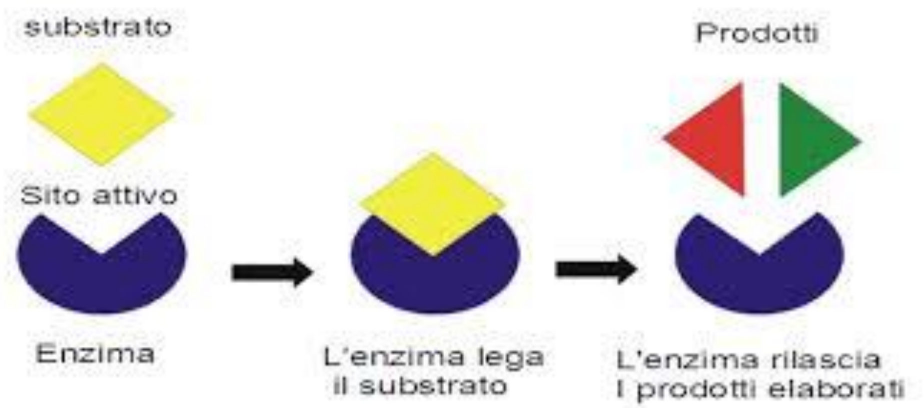
L'idrolisi può avvenire utilizzando:

- Acqua;
- Temperatura;
- Sostanze chimiche (idrolisi chimica);
- Enzimi (idrolisi enzimatica).

Idrolisi chimica: è un processo di idrolisi tramite acido solforico, idrossido di sodio, ecc. e quindi a pH acido o basico ed in genere, ad alta temperatura (> 100 °C). È un ***processo aggressivo*** che punta ad ottenere un prodotto con una maggior quantità di ***amminoacidi totali***, ma che porta con sé molti svantaggi, tra i quali ***amminoacidi non assimilabili in forma destrogira, alta salinità*** ecc…



Idrolisi enzimatica: è un processo di idrolisi delle proteine animali o vegetali che avviene ad opera di ***enzimi specifici*** e selettivi in grado di scindere la catena di amminoacidi in punti specifici. Tale processo avviene all'interno di bioreattori controllati, a bassa temperatura (40-50 °C) e pH vicino alla neutralità. Condizioni ottimali che consentono agli enzimi di idrolizzare il substrato proteico e di conservare gli ***amminoacidi nella loro forma naturale (levogira).***



L'idrolisi enzimatica avviene utilizzando enzimi, che permettono ottenere una racemizzazione nulla e di conseguenza una **elevata presenza di amminoacidi levogiri (biologicamente attivi),** gli unici riconosciuti dalle piante.

Il prodotto finale di questo processo è caratterizzato da una buona stabilità, da una bassa salinità e da una buona miscibilità con tutti i prodotti presenti in commercio.

Un parametro per valutare la qualità di un idrolizzato proteico è il **grado di racemizzazione**, che tiene conto della quantità e della disponibilità degli amminoacidi. La racemizzazione è un **fenomeno naturale che determina una mutazione degli amminoacidi da una forma levogira ad una forma destrogira**.

In natura, ed anche con l'idrolisi enzimatica, gli amminoacidi si trovano sotto forma levogira, (la luce polarizzata avrà un orientamento a sinistra, mentre gli amminoacidi destrogiri si formano a causa dell'aggressività del processo di idrolisi chimica che modifica la forma naturale dell'amminoacido, per il quale, visto al microscopio, la luce polarizzata avrà un orientamento a destra).

Quest'ultimi vengono considerati un indice negativo della qualità di un biostimolante e/o fertilizzante, in quanto gli amminoacidi in forma destrogira non vengono mai assimilati dalla pianta. Inoltre le alte temperature determinano una denaturazione delle molecole organiche presenti nella matrice madre (vitamine, flavonidi, ecc).

- Aumentano la crescita della pianta e la produzione di clorofilla
- Stimolano l'assimilazione dell'N (a livello di espressione genica ed attività enzimatica
- Promuovono la sintesi si composti antiossidanti (es. composti fenolici) e l'accumulo di zuccheri riducenti

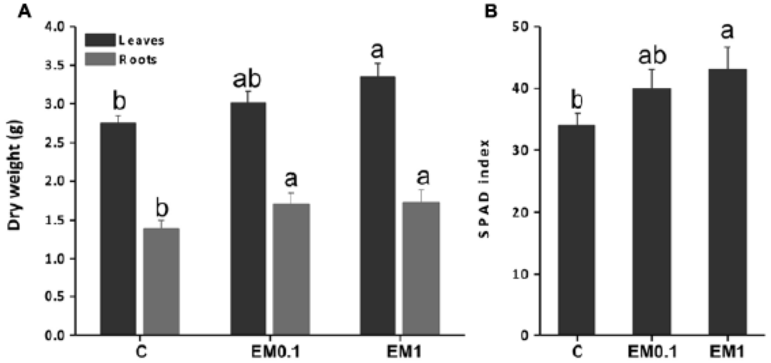
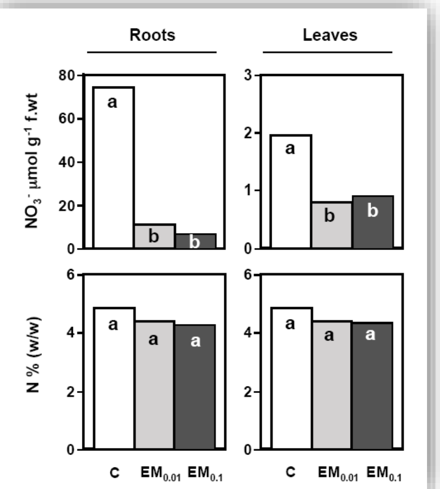
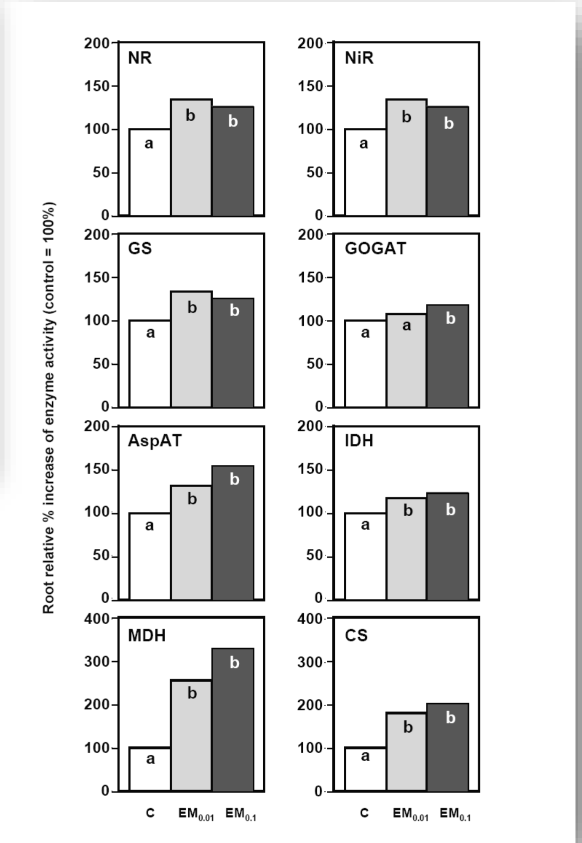
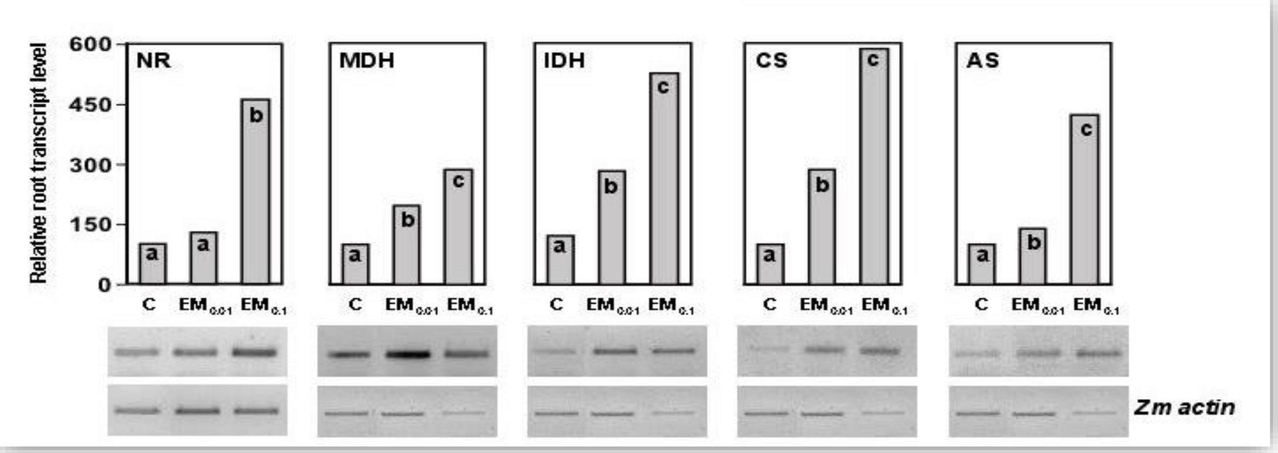


TABLE 3 | Content of soluble sugars (glucose and fructose) and total phenols (TP), and total antioxidant capacity (TAC).

	Glucose	Fructose	TP	TAC
	mg g^{-1} FW		mg GA eq kg^{-1} FW	mg Fe^2 kg^{-1} FW
Leaves				
Control	1.96 ± 0.18b	2.45 ± 0.32c	0.36 ± 0.02b	4.63 ± 0.22b
EM 0.1	2.09 ± 0.33b	1.76 ± 0.47b	0.43 ± 0.03a	6.40 ± 0.31a
EM 1	5.61 ± 0.29a	6.41 ± 0.58a	0.41 ± 0.02a	5.15 ± 0.19a
Roots				
Control	0.51 ± 0.12	0.32 ± 0.10	0.18 ± 0.02a	1.35 ± 0.12a
EM 0.1	1.22 ± 0.21	1.24 ± 0.15	0.23 ± 0.03a	2.13 ± 0.14b
EM 1	1.11 ± 0.19	0.88 ± 0.17	0.19 ± 0.03a	1.82 ± 0.30b

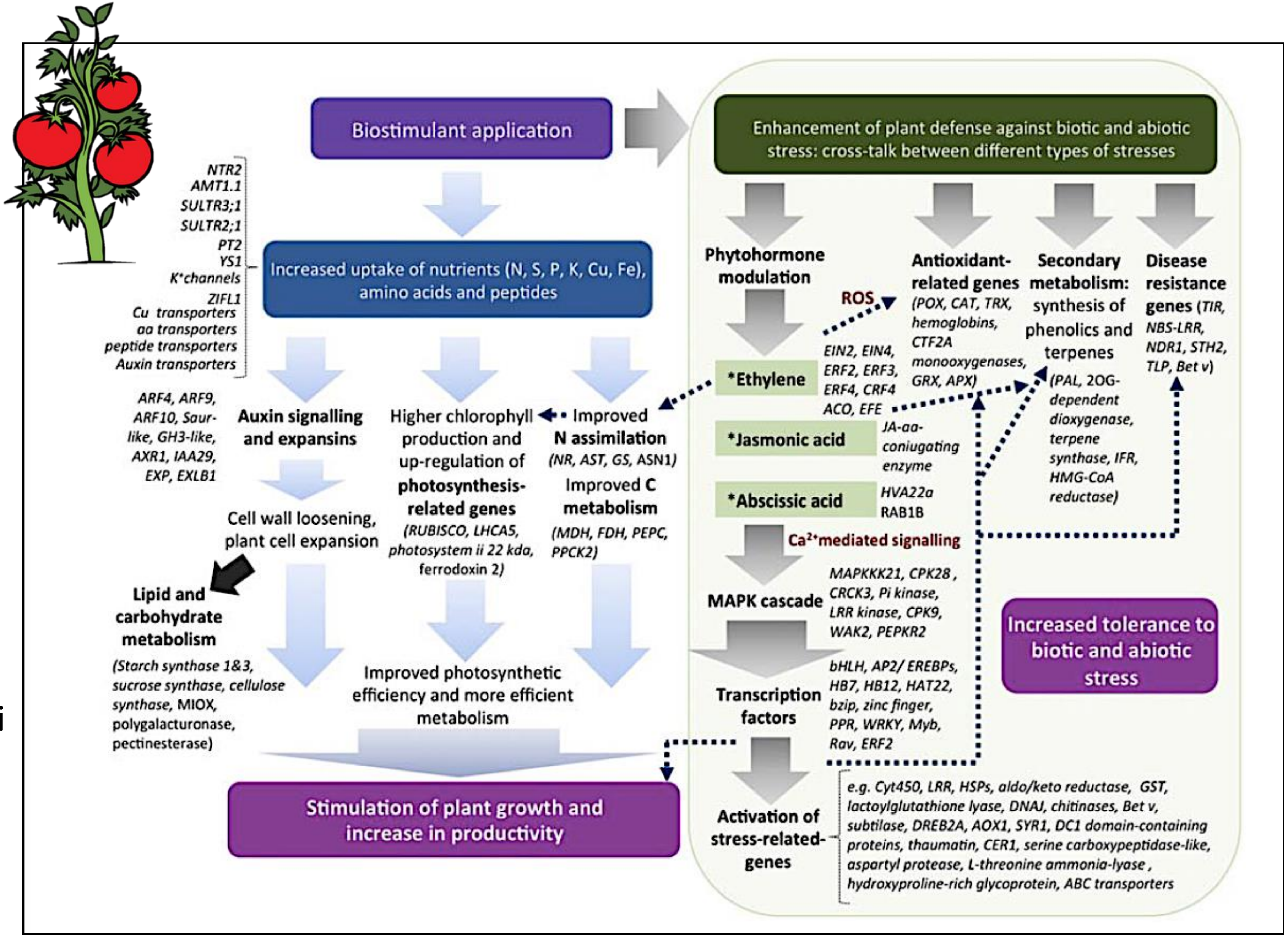






Target principali

- ✓ Meccanismi di difesa delle piante contro stress biotici e abiotici (es. stress salino in mais)
- ✓ Trasportatori di nutrienti, aminoacidi e peptidi
- ✓ Metabolismo dell’N e del C (respirazione cellulare e fotosintesi)
- ✓ Metabolismo degli zuccheri e dei lipidi
- ✓ Stimolazione della crescita attraverso una regolazione di geni coinvolti nel trasporto e nelle vie di segnalazione dell’auxina



Effetti degli idrolizzati proteici evidenziati in diverse specie vegetali

Table 2
Effects of protein hydrolysates (PHs) on horticultural crops.

Crop	Type of PH	PH application mode	Experimental conditions	Effects	References
Banana	Chicken feather derived PH	Root and foliar	Field trial	Early flowering; increased nutrient, chlorophyll content, and proline in leaves; reduced sugars, proteins, amino acids, phenolics and flavonoids in fruits	Gurav and Jadhav (2013)
Corn	PHs from meat flour or alfalfa	Root	Hydroponic system under growth chamber	Increased root and leaf growth, and nitrate reductase and glutamine synthetase activities	Ertani et al. (2009)
Corn	Alfalfa derived PH	Root	Hydroponic system under growth chamber	Increased crop salinity tolerance, nitrogen assimilation and activity of antioxidant systems	Ertani et al. (2013)
Grapevine	PH of distiller's dried grains and carob germ flour	Root	Field trial	Increased total phenolics, and anthocyanin content in grape juice	Parrado et al. (2007)
Grapevine	PHs from soybean or casein	Foliar	Field trial	Up-regulated defense genes encoding pathogenesis-related proteins and the stilbene synthase enzyme; increased resistance to *Plasmopara viticola*	Lachhab et al. (2014)
Grapevine	Plant derived PH	Foliar	Field trial	Increased tolerance to drought, soluble solids, total phenols and anthocyanins in fruits	Boselli et al. (2015)
Kiwifruit	Animal derived PHs with different molecular weights	Foliar	Pot trial	Shoot and root biomass were increased by PH fractions with the lowest molecular weight especially at low rates	Quartieri et al. (2002)
Lettuce	Plant derived PH (Trainer)	Root and foliar	Pot culture under greenhouse using saline and non-saline solution	Increased crop tolerance to salinity, chlorophyll fluorescence, nitrogen and phosphorus content of leaves	Lucini et al. (2015)
Lettuce	Plant derived PH (Trainer)	Root	Hydroponic system with two concentration of nutrient solution	Increased yield, SPAD index, and nitrogen content of leaves	Colla et al. (2013)
Lettuce	Plant derived PH (Aminol 16)	Root and foliar	Greenhouse crop during winter season; foliar and soil application of PH	Increased crop uniformity, and antioxidant activity; reduced nitrates in leaves	Tsouvaltzis et al. (2014)
Lettuce	Animal derived PH (Terra-Sorb Foliar)	Foliar	Pot culture in growth chamber under cold stress conditions	Increased plant fresh weight and stomatal conductance	Botta (2013)
Lily	Animal derived PH and alfalfa derived PH	Foliar	Pot culture under greenhouse conditions	Reduced the length of crop cycle; increased leaf area, diameter of flower buds, and stem and bulb dry weight	De Lucia and Vecchietti (2012)
Olive	Animal derived-PH (Siapton)	Foliar	In vivo and in vitro trials	Increased pollen tube elongation	Viti et al. (1990)
Papaya	Animal derived-PH (Siapton)	Foliar	Field trial	Increased yield	Morales-Pajan and Stall (2003)
Passionfruit	Animal derived PH	Foliar	Nursery	Increased seedling growth	Morales-Pajan and Stall (2004)
Pepper	Alfalfa derived PH	Foliar	Pot culture under greenhouse conditions	Increased fresh weight and number of fruits, and secondary metabolites in fruits	Ertani et al. (2014)
Pepper	Animal derived PH plus micronutrients (Fosfonutren)	Foliar	Pot culture under greenhouse conditions during fall-winter season	Decreased growth, yield and efficiency and utilization of nitrates	Ruiz et al. (2000)
Persimmon	Animal derived PH containing Ca (Stressal)	Root	Field trial under saline conditions	Decreased Cl uptake, leaf necrosis, and leaf water potential	Visconti et al. (2015)
Spinach	Animal derived PH (Siapton)	Foliar	Field trials in spring and autumn seasons using two cultivars	No effect on yield; positive or no effect on dry matter and nitrate content of leaves	Kunicki et al. (2010)
Strawberry	Animal derived PH (Aminoflor)	Foliar	Bag culture under greenhouse conditions	Decreased weight of daughter plants	Lisiecka et al. (2011)
Tomato	Carob germ derived PH	Root	Pot culture under greenhouse condition	Increased plant height, number of flowers, and number of fruits	Parrado et al. (2008)
Tomato	Animal and plant derived PHs	Root and foliar	Hydroponic system with plants grown in Fe-sufficient nutrient solution or in lime-induced Fe deficiency	Growth depression with animal derived PH while plant derived PH enhanced root Fe(III)-chelate reductase activity, chlorophyll concentration, and leaf Fe concentration under lime conditions	Cerdán et al. (2013)
Tomato	Plant derived PH (Trainer)	Root	Soilless culture in growth chamber	Increased rooting and shoot growth	Colla et al. (2014)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Protein hydrolysates as biostimulants in horticulture

Giuseppe Colla[a,*], Serenella Nardi[b], Mariateresa Cardarelli[c], Andrea Ertani[b], Luigi Lucini[d], Renaud Canaguier[e], Youssef Rouphael[f]

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Effetti degli estratti di alghe su alcune specie vegetali

- ✓ Tolleranza a stress salino, siccità e basse temperature (freezing) → regolazione dell'espressione di geni associati alle risposte agli stress
- ✓ Stimolazione della crescita attraverso la modulazione di fitormoni
- ✓ Assorbimento dei nutrienti

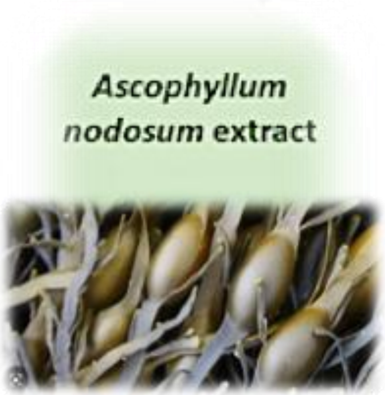
Arabidopsis thaliana

Spinacia oleracea

- ✓ Crescita, resa e qualità nutrizionale; mitigazione stress da siccità
- ✓ Aumento del contenuto di fenoli e antiossidanti
- ✓ Aumento della qualità e della conservazione in post-raccolta

- ✓ Aumento della crescita delle piante e della qualità dei frutti
- ✓ Mitigazione dello stress salino

Solanum lycopersicon



Brassica napa

- ✓ Aumento di crescita e resa
- ✓ Stimolazione del metabolismo di C, N e S

- ✓ Aumento dello sviluppo delle piante e della qualità dei frutti
- ✓ Stimolazione della crescita radicale

Fragaria ananassa

Brassica oleracea

- ✓ Accumulo di isotiocianati, composti fenolici e flavonoidi

- ✓ Morfologia radicale e nutrizione

Zea mays

Vitis vinifera

- ✓ Aumento di crescita e resa
- ✓ Dinamica di maturazione e qualità dei frutti

Azione diretta delle SU sullo sviluppo e il metabolismo vegetale

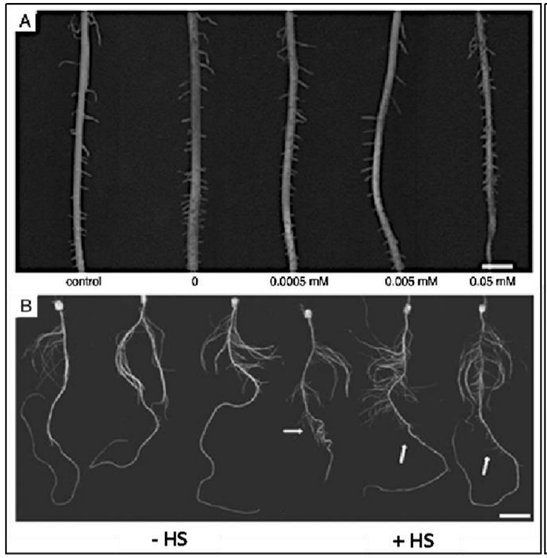
Gli effetti diretti sono attribuiti agli effetti benefici delle SU sulla crescita delle piante mediante l'interazione con le radici.

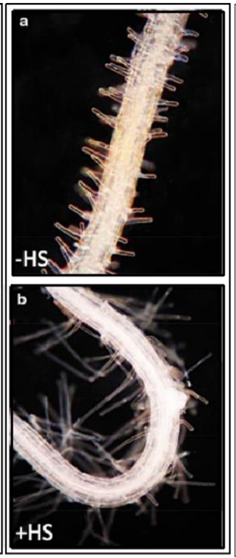
Questi effetti dipendono dalla capacità delle SU di:

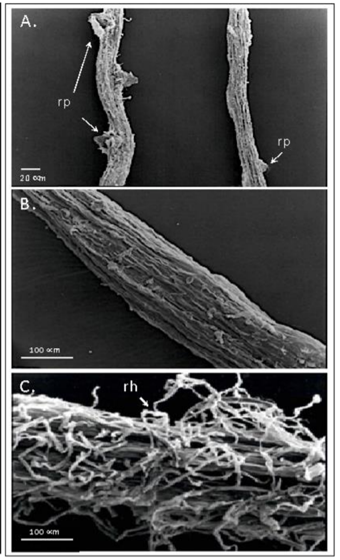
- ✓ aumentare il numero di trasportatori coinvolti nell'assorbimento radicale dei nutrienti e nello stimolare vie metaboliche target all'interno delle piante (SU estratte dal suolo, compost e leonardite)
- ✓ promuovere la crescita delle radici in termini di biomassa e modificare i tratti radicali associati all'acquisizione dei nutrienti

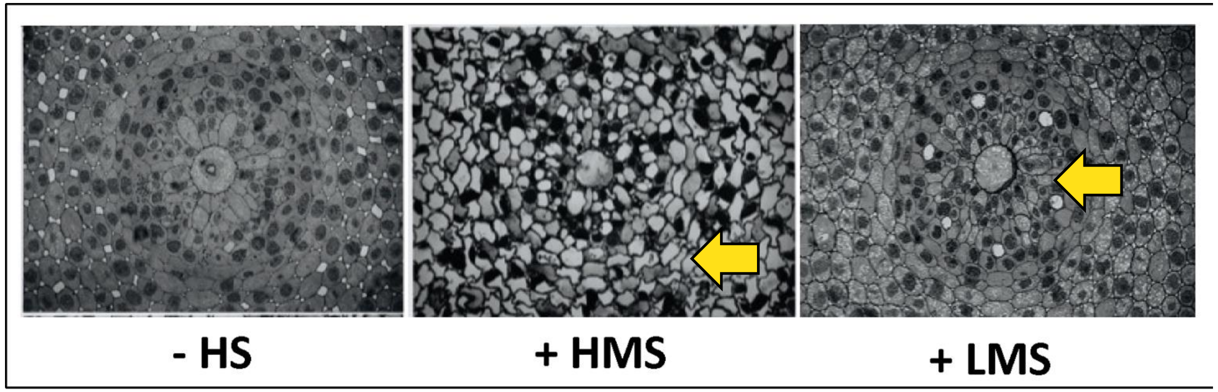
Regolazione a livello trascrizionale e post-trascrizionale (trasportatori di nutrienti, enzimi chiave del metabolismo, pompe protoniche)

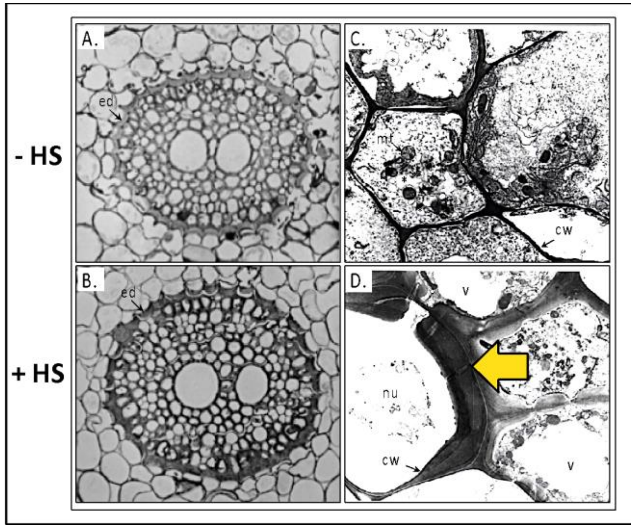
Effetti diretti e meccanismi d'azione: sviluppo radicale











- ✓ **Anticipo della differenziazione cellulare**
- ✓ **Ispessimento delle pareti cellulari** ciò favorisce il trasporto dell'acqua e dei nutrienti, barriera fisica per attacchi di patogeni
- ✓ **Allungamento radicale e aumento della produzione di biomassa ROS**
- ✓ **Incremento del numero di peli radicali e di radici laterali** azione ormono (IAA)-simile

Effetti diretti e meccanismi d'azione delle SU: sviluppo radicale

L'applicazione di SU alla rizosfera o alle foglie determina generalmente miglioramenti significativi in termini di:

✓ **Assorbimento radicale di N**

✓ **Espressione genica del trasportatore del nitrato**

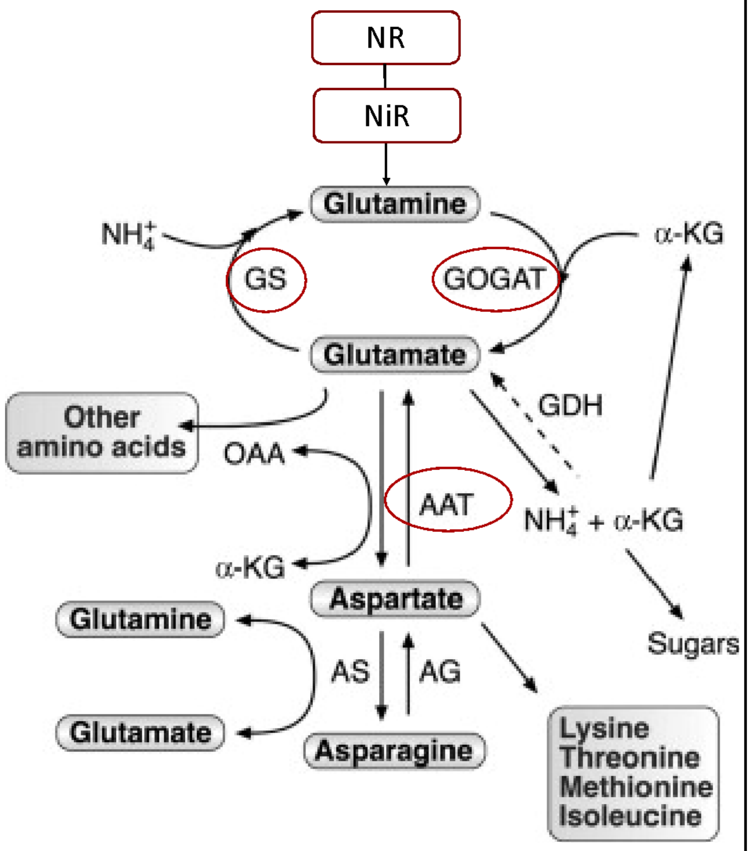
✓ **Assimilazione di N all'interno della pianta**

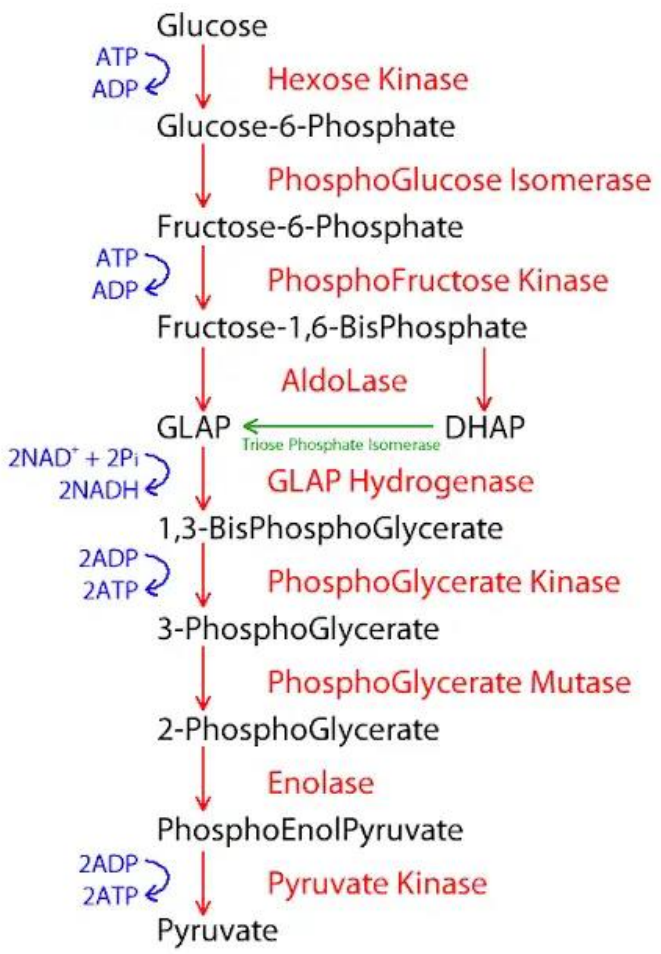
- L'aumento dell'assorbimento di N è correlato con una maggiore attività ed espressione genica dell'H^+-ATPasi della membrana radicale e con l'aumento della traslocazione da radice a germoglio delle citochinine attive

- I gruppi funzionali aromatici e alifatici delle SU sono responsabili di un maggiore assorbimento di N

Effetti diretti delle sostanze umiche sul metabolismo delle piante: principali target metabolici

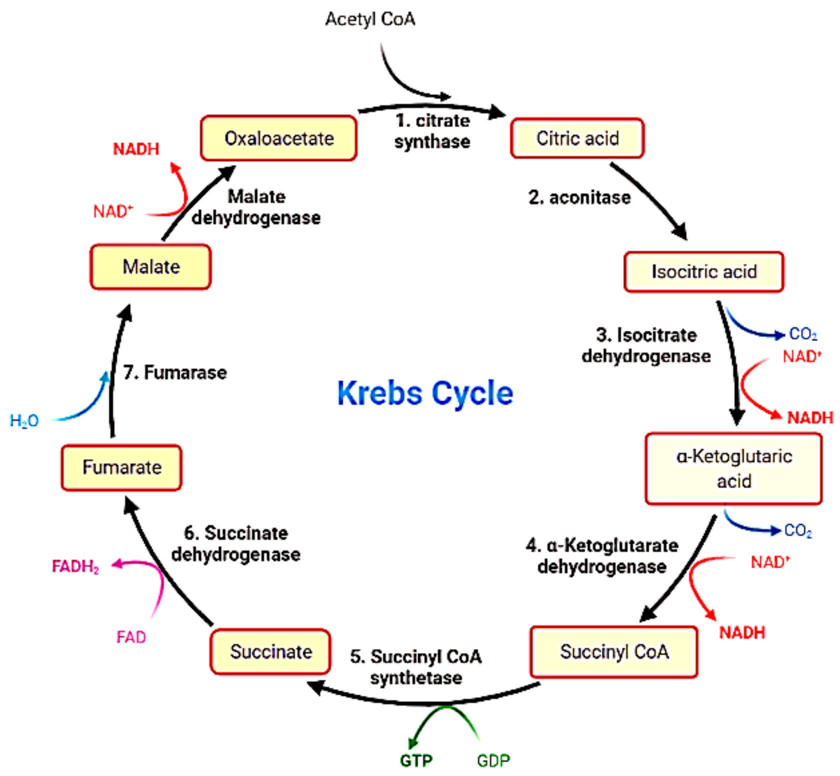
Assimilazione dell’N





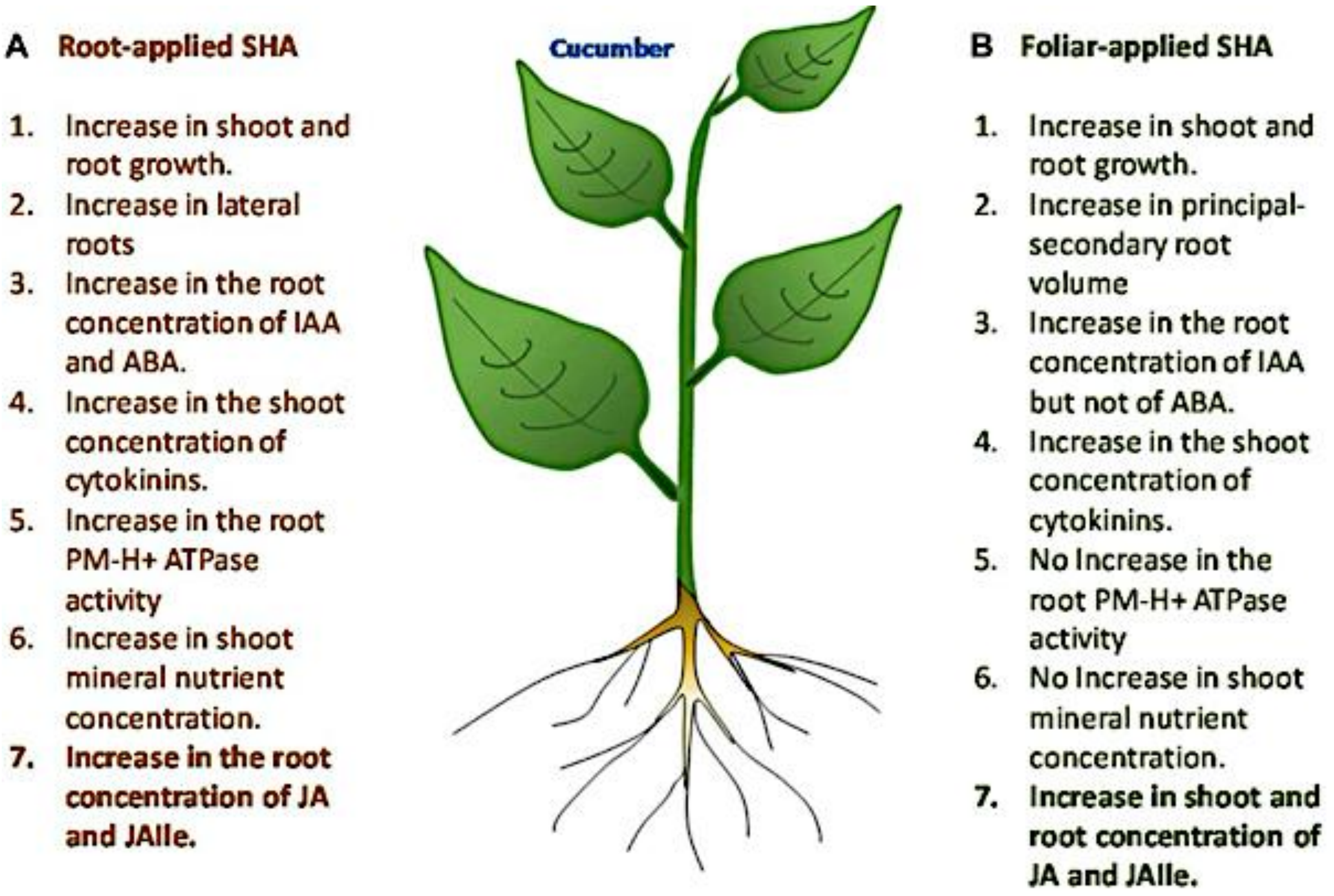
Simplified Glycolysis diagram. Molecule names contain extra capitals to illustrate components. 21/02/2010 followchemistry.wordpress.com

Metabolismo respiratorio: glicolisi e ciclo di Krebs



Effetti diversi e comuni indotti da SU applicate Sulle foglie e radici

- ✓ Quando le SU vengono applicate per via fogliare, verranno meno i meccanismi relativi alla mobilizzazione dei nutrienti nel suolo derivanti dalla capacità di complessazione dei metalli delle SU
- ✓ Le SU applicate alle foglie non determinano l’aumento dell'attività dell'H^+-ATPasi, né l’aumento del contenuto di nutrienti nella parte aerea.
- ✓ L'aumento del contenuto di ormoni coinvolti nei processi di difesa e localizzato nell’organo in cui viene effettuata l’applicazione di SU

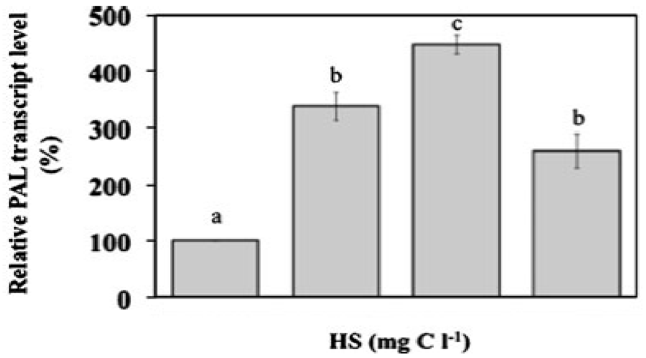


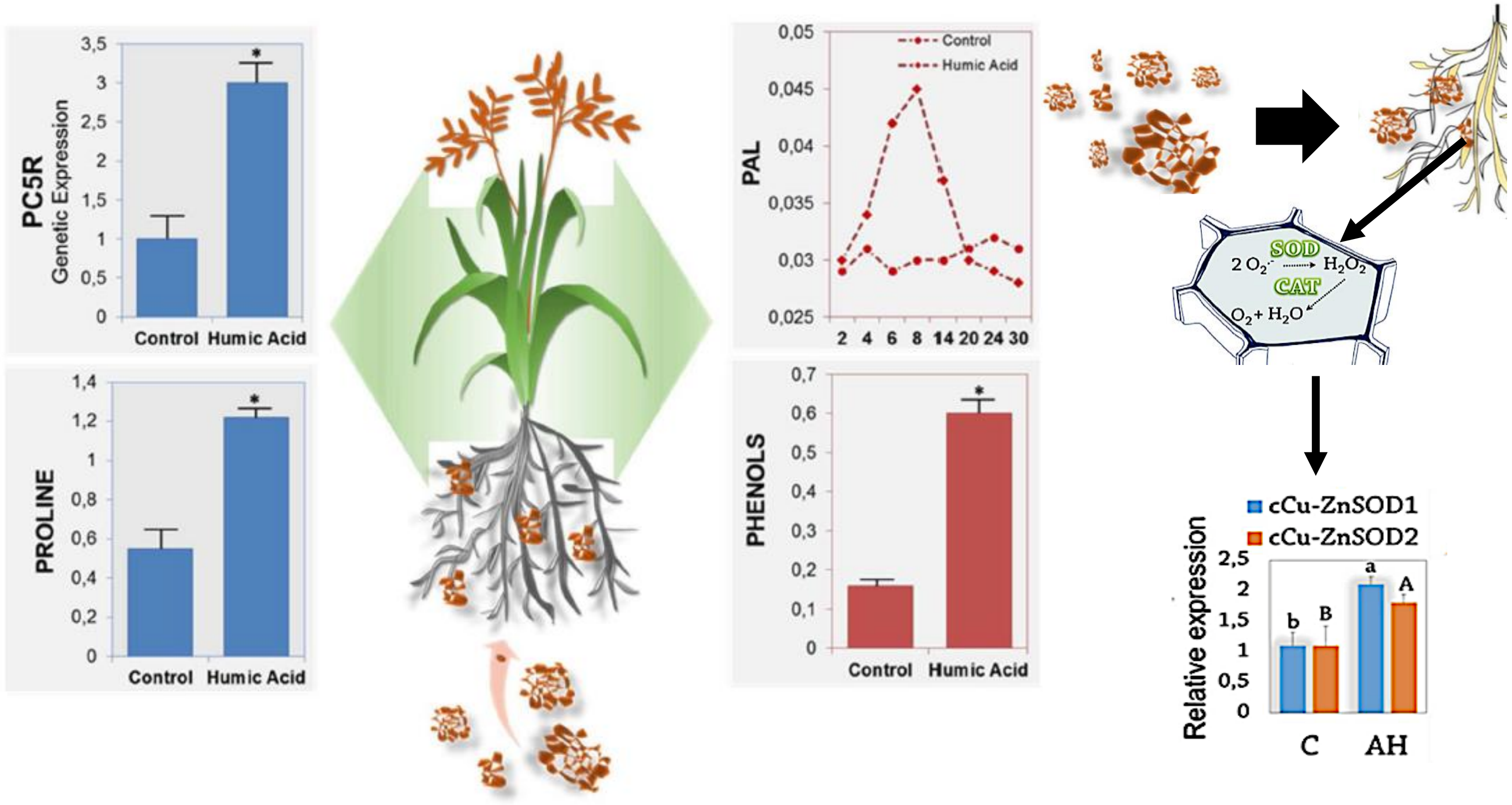
Le SU possono migliorare i sistemi di difesa delle piante contro stress abiotici e biotici

Aumentano la sintesi e l'accumulo di vari metaboliti secondari (composti fenolici, fitoalessine, prolina, etc.) + effetto positivo sull'attività e sull'espressione genica di enzimi antiossidanti

Table 4 Phenylalanine ammonia-lyase (PAL), tyrosine ammonia-lyase (TAL) activities, total phenolic acid and flavonoid content in *Zea mays* leaves. Plants were treated for 48 h with high molecular weight humic substances (HS) at the indicated concentrations[a]

HS[a]	PAL[c]	TAL[d]	Phenolic acids[e]	Flavonoids[f]
0.0	9.70±0.12 c[b]	0.13±0.06 d	1.03±0.05 d	0.162±0.002 c
0.5	10.67±0.35 b	0.58±0.06 c	1.25±0.13 c	0.186±0.003 b
1.0	13.58±0.41 a	0.83±0.02 a	1.49±0.07 a	0.200±0.005 a
2.0	13.09±0.31a	0.75±0.02 b	1.34±0.05 b	0.188±0.007 b





Idrolizzati proteici (PH)

- ✓ Prodotti di origine diversa
- ✓ **Generati attraverso idrolisi termica, chimica o enzimatica**

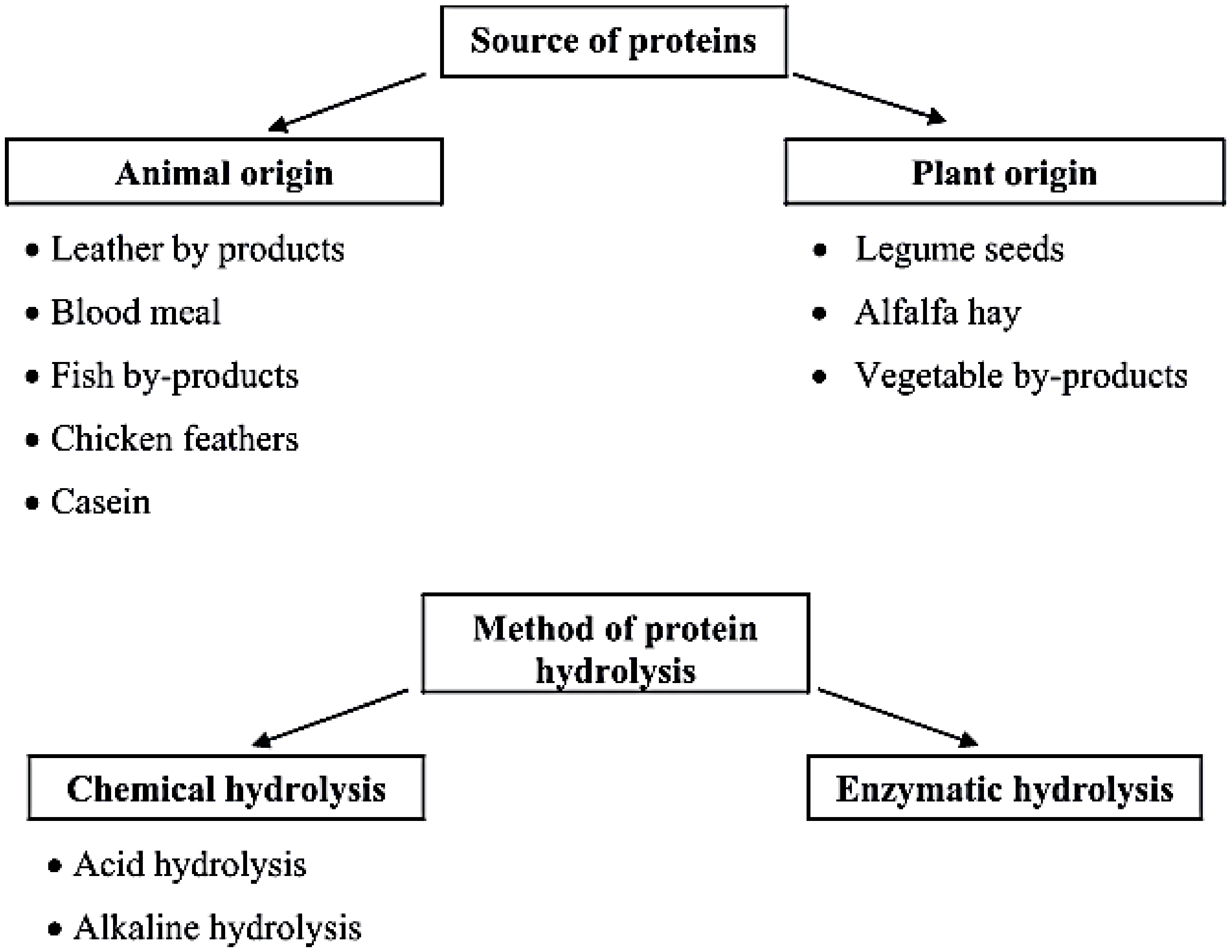


Fig. 1. Classification criteria of protein hydrolysates on the basis of protein source and the method of protein hydrolysis used in the production process.

- Aumentano la crescita della pianta e la produzione di clorofilla
- Stimolano l'assimilazione dell'N (a livello di espressione genica ed attività enzimatica
- Promuovono la sintesi si composti antiossidanti (es. composti fenolici) e l'accumulo di zuccheri riducenti

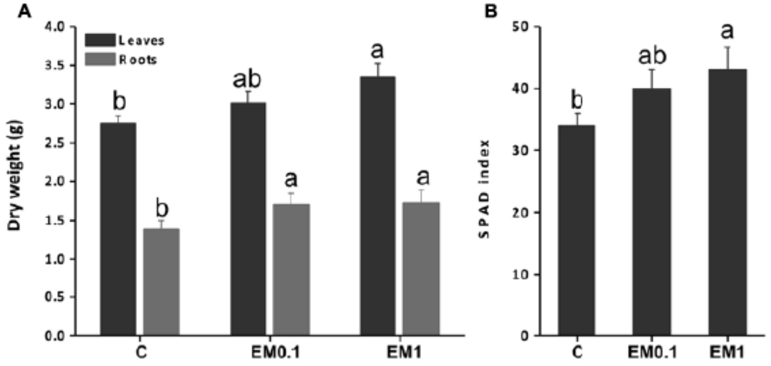
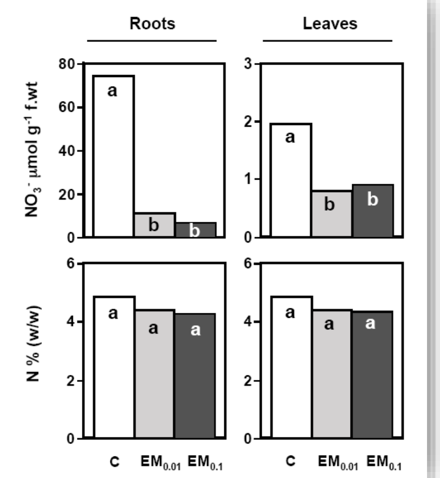
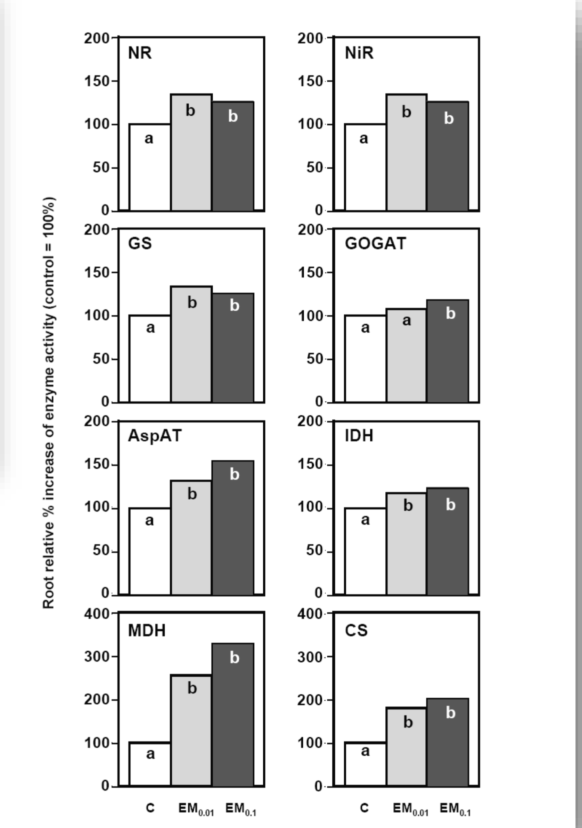
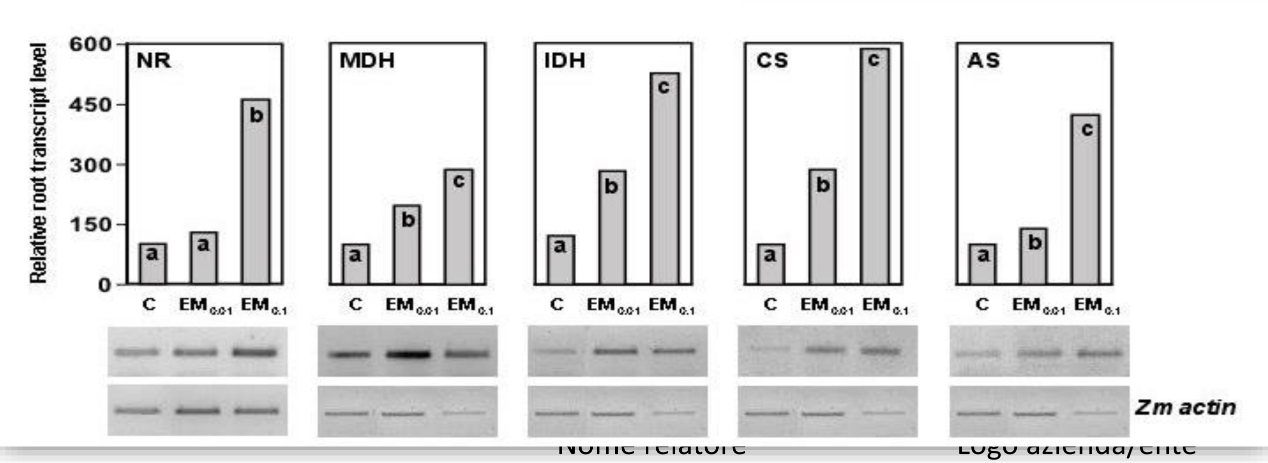


TABLE 3 | Content of soluble sugars (glucose and fructose) and total phenols (TP), and total antioxidant capacity (TAC).

	Glucose	Fructose	TP	TAC
	mg g^{-1} FW		mg GA eq kg^{-1} FW	mg Fe^{2} kg^{-1} FW
Leaves				
Control	1.96 ± 0.18b	2.45 ± 0.32c	0.36 ± 0.02b	4.63 ± 0.22b
EM 0.1	2.09 ± 0.33b	1.76 ± 0.47b	0.43 ± 0.03a	6.40 ± 0.31a
EM 1	5.61 ± 0.29a	6.41 ± 0.58a	0.41 ± 0.02a	5.15 ± 0.19a
Roots				
Control	0.51 ± 0.12	0.32 ± 0.10	0.18 ± 0.02a	1.35 ± 0.12a
EM 0.1	1.22 ± 0.21	1.24 ± 0.15	0.23 ± 0.03a	2.13 ± 0.14b
EM 1	1.11 ± 0.19	0.88 ± 0.17	0.19 ± 0.03a	1.82 ± 0.30b

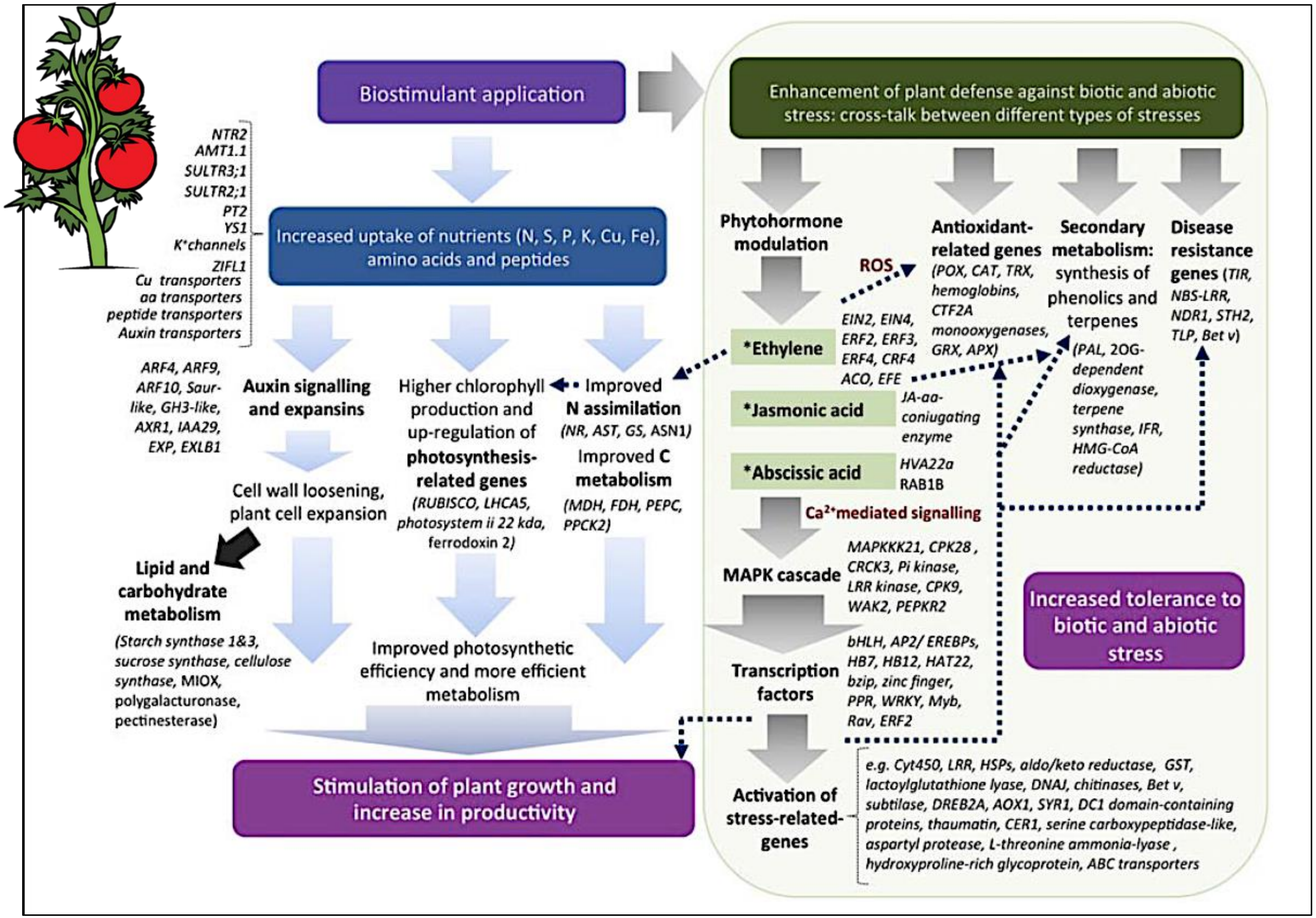






Target principali

- ✓ Meccanismi di difesa delle piante contro stress biotici e abiotici (es. stress salino in mais)
- ✓ Trasportatori di nutrienti, aminoacidi e peptidi
- ✓ Metabolismo dell’N e del C (respirazione cellulare e fotosintesi)
- ✓ Metabolismo degli zuccheri e dei lipidi
- ✓ Stimolazione della crescita attraverso una regolazione di geni coinvolti nel trasporto e nelle vie di segnalazione dell’auxina



Effetti degli idrolizzati proteici evidenziati in diverse specie vegetali

Table 2
Effects of protein hydrolysates (PHs) on horticultural crops.

Crop	Type of PH	PH application mode	Experimental conditions	Effects	References
Banana	Chicken feather derived PH	Root and foliar	Field trial	Early flowering; increased nutrient, chlorophyll content, and proline in leaves; reduced sugars, proteins, amino acids, phenolics and flavonoids in fruits	Gurav and Jadhav (2013)
Corn	PHs from meat flour or alfalfa	Root	Hydroponic system under growth chamber	Increased root and leaf growth, and nitrate reductase and glutamine synthetase activities	Ertani et al. (2009)
Corn	Alfalfa derived PH	Root	Hydroponic system under growth chamber	Increased crop salinity tolerance, nitrogen assimilation and activity of antioxidant systems	Ertani et al. (2013)
Grapevine	PH of distiller's dried grains and carob germ flour	Root	Field trial	Increased total phenolics, and anthocyanin content in grape juice	Parrado et al. (2007)
Grapevine	PHs from soybean or casein	Foliar	Field trial	Up-regulated defense genes encoding pathogenesis-related proteins and the stilbene synthase enzyme; increased resistance to *Plasmopara viticola*	Lachhab et al. (2014)
Grapevine	Plant derived PH	Foliar	Field trial	Increased tolerance to drought, soluble solids, total phenols and anthocyanins in fruits	Boselli et al. (2015)
Kiwifruit	Animal derived PHs with different molecular weights	Foliar	Pot trial	Shoot and root biomass were increased by PH fractions with the lowest molecular weight especially at low rates	Quartieri et al. (2002)
Lettuce	Plant derived PH (Trainer)	Root and foliar	Pot culture under greenhouse using saline and non-saline solution	Increased crop tolerance to salinity, chlorophyll fluorescence, nitrogen and phosphorus content of leaves	Lucini et al. (2015)
Lettuce	Plant derived PH (Trainer)	Root	Hydroponic system with two concentration of nutrient solution	Increased yield, SPAD index, and nitrogen content of leaves	Colla et al. (2013)
Lettuce	Plant derived PH (Aminol 16)	Root and foliar	Greenhouse crop during winter season; foliar and soil application of PH	Increased crop uniformity, and antioxidant activity; reduced nitrates in leaves	Tsouvaltzis et al. (2014)
Lettuce	Animal derived PH (Terra-Sorb Foliar)	Foliar	Pot culture in growth chamber under cold stress conditions	Increased plant fresh weight and stomatal conductance	Botta (2013)
Lily	Animal derived PH and alfalfa derived PH	Foliar	Pot culture under greenhouse conditions	Reduced the length of crop cycle; increased leaf area, diameter of flower buds, and stem and bulb dry weight	De Lucia and Vecchietti (2012)
Olive	Animal derived-PH (Siapton)	Foliar	In vivo and in vitro trials	Increased pollen tube elongation	Viti et al. (1990)
Papaya	Animal derived-PH (Siapton)	Foliar	Field trial	Increased yield	Morales-Pajan and Stall (2003)
Passionfruit	Animal derived PH	Foliar	Nursery	Increased seedling growth	Morales-Pajan and Stall (2004)
Pepper	Alfalfa derived PH	Foliar	Pot culture under greenhouse conditions	Increased fresh weight and number of fruits, and secondary metabolites in fruits	Ertani et al. (2014)
Pepper	Animal derived PH plus micronutrients (Fosfonutren)	Foliar	Pot culture under greenhouse conditions during fall-winter season	Decreased growth, yield and efficiency and utilization of nitrates	Ruiz et al. (2000)
Persimmon	Animal derived PH containing Ca (Stressal)	Root	Field trial under saline conditions	Decreased Cl uptake, leaf necrosis, and leaf water potential	Visconti et al. (2015)
Spinach	Animal derived PH (Siapton)	Foliar	Field trials in spring and autumn seasons using two cultivars	No effect on yield; positive or no effect on dry matter and nitrate content of leaves	Kunicki et al. (2010)
Strawberry	Animal derived PH (Aminoflor)	Foliar	Bag culture under greenhouse conditions	Decreased weight of daughter plants	Lisiecka et al. (2011)
Tomato	Carob germ derived PH	Root	Pot culture under greenhouse condition	Increased plant height, number of flowers, and number of fruits	Parrado et al. (2008)
Tomato	Animal and plant derived PHs	Root and foliar	Hydroponic system with plants grown in Fe-sufficient nutrient solution or in lime-induced Fe deficiency	Growth depression with animal derived PH while plant derived PH enhanced root Fe(III)-chelate reductase activity, chlorophyll concentration, and leaf Fe concentration under lime conditions	Cerdán et al. (2013)
Tomato	Plant derived PH (Trainer)	Root	Soilless culture in growth chamber	Increased rooting and shoot growth	Colla et al. (2014)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Protein hydrolysates as biostimulants in horticulture

Giuseppe Colla[a,*], Serenella Nardi[b], Mariateresa Cardarelli[c], Andrea Ertani[b], Luigi Lucini[d], Renaud Canaguier[e], Youssef Rouphael[f]

Estratti di alghe

Composizione chimica e biologica

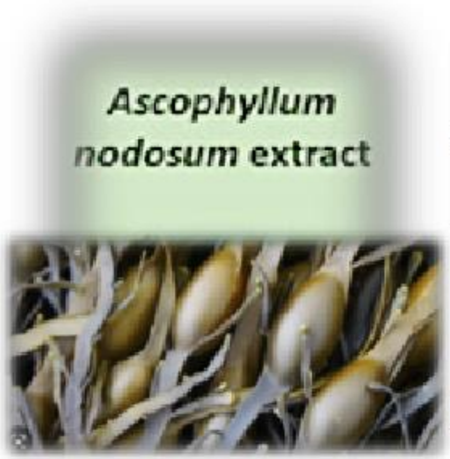
Esperimenti *in planta* in condizioni controllate ← Determinazione del livello di tossicità e screening iniziali di efficacia (via test di germinazione) ← Centrifugazione e filtrazione ← Metodi di estrazione

Analisi omiche

Comprensione dei meccanismi d'azione e ottimizzazione delle procedure di estrazione → Validazione attraverso prove in serra e in campo → Formulazione del prodotto

- Estrazione in acqua (bs. arricchiti in composti con attività ormono-simile)
- Acid hydrolysis (bs. arricchiti in polisaccaridi solfatati contenenti fucosio)
- Idrolisi alcalina (bs. arricchiti in composti nuovi)
- Estrazione in microonde(MAE) (bs. arricchiti in fucoidani, alginato di sodio, zuccheri e composti fenolici
- Estrazione mediante ultrasuoli (UAE) (bs. arricchiti in laminarina e altri composti bioattivi)
- Estrazione in presenza di enzimi (Evita problemi di solubilità in acqua)
- Estrazione supercritica (protegge il materiale algale dalla degradazione termica o biochimica dei composti bioattivi)

Effetti degli estratti di alghe su alcune specie vegetali



- Tolleranza a stress salino, siccità e basse temperature (freezing) regolazione dell'espressione di geni associati alle risposte agli stress
- Stimolazione della crescita attraverso la modulazione di fitormoni
- Assorbimento dei nutrienti

Arabidopsis thaliana

Spinacia oleracea

- Crescita, resa e qualità nutrizionale; mitigazione stress da siccità
- Aumento del contenuto di fenoli e antiossidanti
- Aumento della qualità e della conservazione in post-raccolta

- Aumento della crescita delle piante e della qualità dei frutti
- Mitigazione dello stress salino

Solanum lycopersicon

Brassica napa

- Aumento di crescita e resa
- Stimolazione del metabolismo di C, N e S

- Aumento dello sviluppo delle piante e della qualità dei frutti
- Stimolazione della crescita radicale

Fragaria ananassa

Brassica oleracea

- Accumulo di isotiocianati, composti fenolici e flavonoidi

- Morfologia radicale e nutrizione

Zea mays

Vitis vinifera

- Aumento di crescita e resa
- Dinamica di maturazione e qualità dei frutti

Extract	Crop	Function	References
GA14® (Goemar, France)	*Spinacia oleracea*	Foliar spray improved total fresh biomass	Cassan et al., 1992
Maxicrop® Original	Tomato	Higher chlorophyll content in sprayed plants	Whapham et al., 1993
Maxicrop®	*Capsicum annuum*	Improved yield and quality	Eris et al., 1995
Goemar®	*Citrus unshiu*	Early maturation of fruit	Fornes et al., 1995
A. nodosum extract	Kiwi fruit	Improved fruit growth, weight, and maturation	Chouliaras et al., 1997
A. nodosum extract	Tomato, dwarf French bean, wheat, barley, maize	Enhanced leaf chlorophyll level	
Acadian® (Acadian Seaplants)	*Vitis vinifera*	Improved yield and fruit quality	Norrie et al., 2002
Acadian® (Acadian Seaplants)	*Poa pratensis*	Improved shelf life and transplant rooting	Zhang et al., 2003
Maxicrop®, Proton®, Algipower®	*Vitis vinifera*	Improved copper uptake of grapevine	Turan and Köse, 2004
Goemar®	Clementine Mandarin and Navelina Orange	Increased productivity and yield	Fornes et al., 2002
A. nodosum extract	*Arabidopsis thaliana*	Improved plant growth by modulation of concentration and localization of auxin	Rayorath et al., 2008
A. nodosum extract	*Hordeum vulgare*	Induced gibberellic-acid-independent amylase activity in barley and promoted seed germination	Rayorath et al., 2008
Goëmar BM 86®	Apple	Improved the fruit quality of apple and have high nitrogen content	Basak, 2008
Acadian® Marine Plant Extract Powder (AMPEP)	*Kappaphycus striatum*	AMPEP improves micro-propogation	Hurtado et al., 2009
A. nodosum extract	*Olea europaea*	Showed increased tree productivity and improved their nutrition status and oil quality parameters	Chouliaras et al., 2009
Alge®	*Citrullus lanatus*	Application of extract showed increased growth parameters and yield responses	Abdel-Mawgoud et al., 2010

Actiwave®	Strawberry	Increases fruit yield and quality and acts as iron chelator	Spinelli et al., 2010
Acadian® (Acadian Seaplants)	*Spinacia oleracea*	Enhances phenolic antioxidant content of Spinach	Fan et al., 2011
AMPEP	*Ulva lactuca*	Reduces ionic liquid induced oxidative stress in *Ulva lactuca*	Kumar et al., 2013
A. nodosum extract	*Medicago sativa*	Improves root colonization of rhizobial symbionts	Khan et al., 2012
A. nodosum extract	Strawberry	Improved plant growth, fruit quality and microbial growth	Alam et al., 2013
Super Fifty®, Ecoelicitor®	Lettuce; Oilseed rape	Enhanced plant growth and tolerance to biotic and biotic stresses	Guinan et al., 2012
Acadian® (Acadian Seaplants)	*Spinacia oleracea*	Improved yield and nutritional quality	Fan et al., 2013
Acadian® (Acadian Seaplants)	*Spinacia oleracea*	Improves phenolics and antioxidant content of spinach	Fan et al., 2013
Alga Special (AS)	*Vitis vinifera*	Improved vegetative growth	Popescu and Popescu, 2014
AZAL5	*Brassica napus*	Promotes plant growth and nutrient uptake	Jannin et al., 2013
AlgaeGreen®	*Brassica oleracea*	Enhanced biosynthesis of secondary metabolites	Lola-Luz et al., 2013
Acadian® (Acadian Seaplants)	*Spinacia oleracea*	Preharvest ANE application enhanced post-harvest storage quality of spinach	Fan et al., 2014
Acadian® (Acadian Seaplants)	Carrot	Promote plant growth and root yield in carrot associated with increased root-zone soil microbial activity	Alam et al., 2014
Stella Maris™	*Calibrachoa hybrida*	Increased biosynthesis of secondary metabolites and enhanced antibacterial and antifungal properties of *C. hybrida* extract	Elansary et al., 2016a
A. nodosum extract	*Vitis vinifera*	Improved growth, yield, berry quality attributes, and leaf nutrient content of grapevines	Sabir et al., 2014
Premium liquid seaweed	*Allium cepa*	Improved vegetative growth and yield of onion	Hidangmayum and Sharma, 2017
Seaweed extract	*Zea mays*	Promotes root morphology and plant nutrition	Ertani et al., 2018
Acadian® (Acadian Seaplants)	*Vitis vinifera*	Foliar spray has a positive effect on ripening dynamics and fruit quality	Frioni et al., 2018
Rygex®, Super fifty®	*Solanum lycopersicum*	Increased plant growth and fruit quality and mitigates salinity stress in tomato plants	Di Stasio et al., 2018
Seaweed extract	*Spinacia oleracea*	Improved growth, quality, and nutritional value of spinach grown under drought conditions	Xu and Leskovar, 2015
Seasol®	*Fragaria ananassa*	Increased growth response of strawberry root	Mattner et al., 2018

REVIEW ARTICLE
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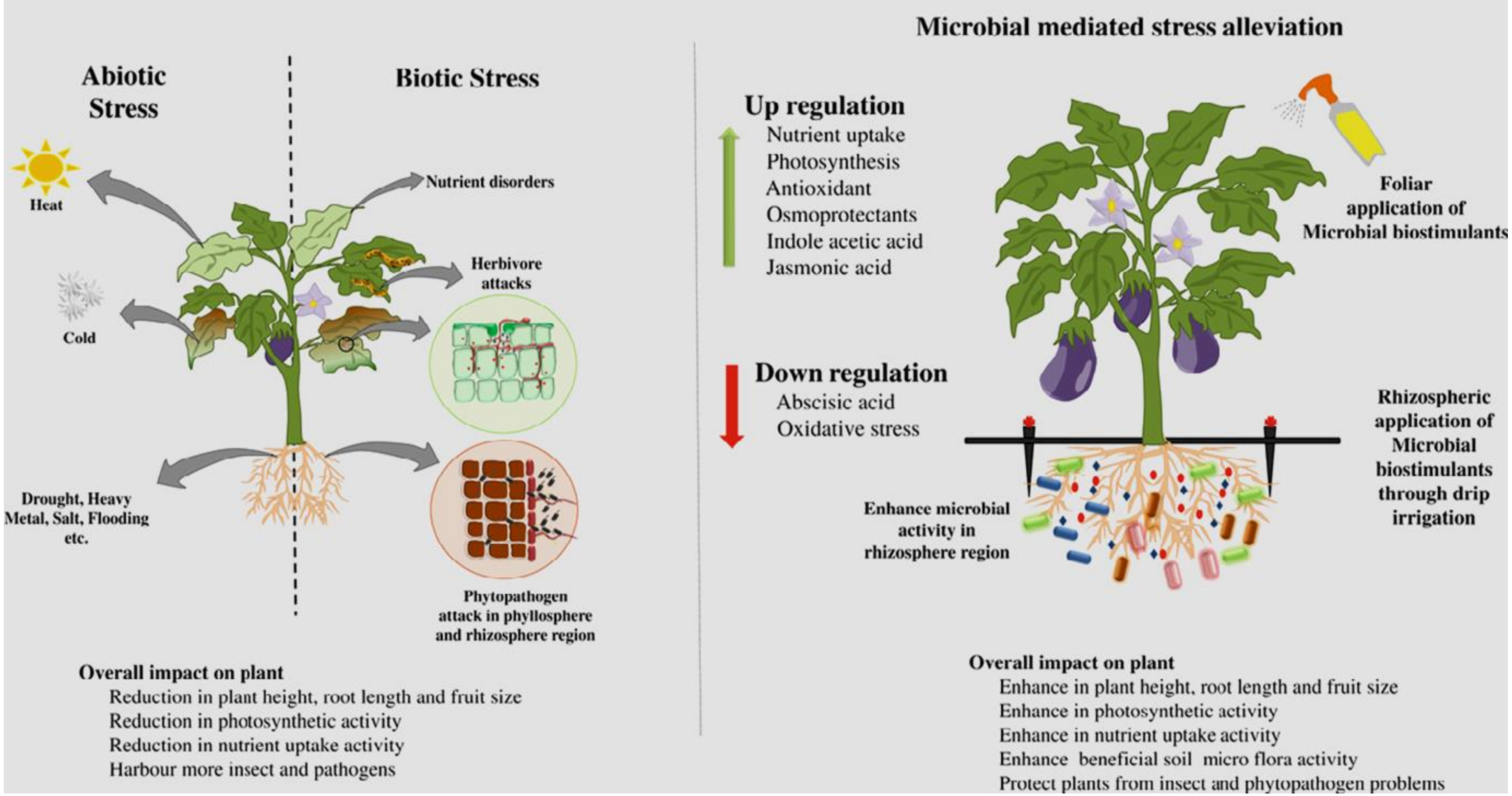
Ascophyllum nodosum-Based Biostimulants: Sustainable Applications in Agriculture for the Stimulation of Plant Growth, Stress Tolerance, and Disease Management

Pushp Sheel Shukla[1], Emily Grace Mantin[1], Mohd Adil[1], Sruti Bajpai[1], Alan T. Critchley[2] and Balakrishnan Prithiviraj[1*]

Biostimolanti microbici

PGPB (*Azotobacter, Azospirillum**)

Mycorrhizal fungi AMV (e.g.



*** Azospirillum**:

- Produce ormoni che stimolano la crescita radicale
- Rende più disponibili N & P
- Fissazione di N
- Produce antiossidanti

*** AMV + estratti di alghe**:

- Fioritura accelerata
- Aumenta il numero dei fiori
- Aumenta la colonizzazione microbica

Table 2. Effect of selected biostimulants on quality of fruit and vegetables.

Biostimulant	Dose	Plant	Type of Claims	Benefitial Effect
Humic and fulvic acids	500 g/100 L	Apricot (*Prunus armeniaca* L.)	Yield	Increase by 75% in the second year compared to the control [8]
Carboxylic acids	200 mL/100 L	Apricot (*Prunus armeniaca* L.)	Yield	Increase 58% in the second year compared to the control [8]
All amino acids	3 L/ha	Mango (*Mangifera indica*)	Yield	Increase by 18% compared to the control [32]
6-benzyladenine	100 mg/L	Blueberry (*Vaccinium corymbosum* L.)	Yield	Increase by 0.5 kg/tree in the first year of using the biostimulant compared to the control [37]
α-naphthaleneacetic acid and 6-benzyladenine	100 mg/L (α-naphthaleneacetic acid) 20 mg/L (6-benzyladenine)	Blueberry (*Vaccinium corymbosum* L.)	Yield	Increase by 1 kg/tree in the second year of using the biostimulant compared to the control [37]
Humic acids	3g/L	Cucumber (*Cucumis sativus* L.)	Diameter	Increase in vegetable diameter by 1.23 cm (first season) and 1.55 cm (second season) compared to the control [52]
Nitrogen, amino acids, auxins	0.45 cm/L	Cucumber (*Cucumis sativus* L.)	Length	Lengthening vegetables by 3.85 cm (first season) and 3.49 cm (second season) compared to the control [52]
Biostimulant	**Dose**	**Plant**	**Type of Claims**	**Benefitial Effect**
Carboxylic acids	200 mL/100 L	Apricot (*Prunus armeniaca* L.)	Diameter	Increase diameter by 2.6 mm (second growing season) compared to the control [8]
Arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* C7	The bacterial inoculum was replicated, watering each plant with 200 mL of bacterial suspension (density about 108 colony forming unit CFU/mL)	Tomato (*Solanum lycopersicum* L.)	Weight	Increase in tomato fruit mass by 6.9 g compared to control [53]
6-benzyladenine	100 mg/L	Blueberry cv. Duke and Bluecrop	Weight	Increase in the weight of blueberry fruit by about 32.4% (first season) and 33.6% (second season) for the blueberry cultivar Duke and 43.5% (first season) and 33.1% (second season) for the blueberry cultivar Bluecrop compared to the control [53]
α-naphthaleneacetic acid	20mg/L	Blueberry cv. Duke and Bluecrop	Weight	Increased the weight of the blueberry cultivar Duke fruit by 41.9% (first season) and 20.0% (second season) and the blueberry cultivar Bluecrop by 55.0% (first season) and 25.4% (second season) compared to the control [37]
Humic Acids	3g/L	Cucumber (*Cucumis sativus* L.)	Length	Increase in vegetable diameter by 1.23 cm (first season) and 1.55 cm (second season) compared to the control [52]
Nitrogen, amino acids, auxins	0.45 cm/L	Cucumber (*Cucumis sativus* L.)	Length	Lengthening vegetables by 3.85 cm in the first and 3.49 cm in the second growing season compared to the control [52]

Table 2. *Cont.*

Carboxylic acids	200 mL/100 L	Apricot (*Prunus armeniaca* L.)	Diameter	Widening of fruit by 2.6 mm on average in the second growing season compared to the control [8]
Arbuscular mycorrhizal fungi and *P. fluorescent* C7	The bacterial inoculum was replicated, watering each plant with 200 mL of bacterial suspension (density about 108 CFU/mL)	Tomato (*Solanum lycopersicum* L.)	Weight	Increase in tomato fruit mass by 6.9 g compared to control [53]
6-Benzyladenine	100 mg/L	Blueberry cv. Duke and Bluecrop	Weight	Increase in the weight of blueberry fruit by about 32.4% (first season) and 33.6% (second season) for the blueberry cultivar Duke and 43.5% (first season) and 33.1% (second season) for the blueberry cultivar Bluecrop compared to the control [53]
α-naphthaleneacetic acid	20 mg/L	Blueberry cv. Duke and Bluecrop	Weight	Increase in weight of the blueberry cultivar Duke fruit by 41.9% (first season) and 20.0% (second season) and the blueberry cultivar Bluecrop by 55.0% (first season) and 25.4% (second season) compared to the control [46]
Biostimulant including, among others, nitrogen, amino acids, auxins	0.45 cm/L	Cucumber (*Cucumis sativus* L.)	Plant height	Increase in plant height of 14.5 cm (in the first growing season) and 19.75 cm (in the second growing season) in comparison with the control plants [52]
Moringa oleifera leaf extract	3% treatments were replicated three times	Pumpkin (*Cucurbita pepo* L.)	Chlorophyll content	34.6% increase in the chlorophyll content compared to the control [50]
Salicylic acid–chitosan nanoparticles	(concentration 0.01%–0.16%)	Maize CV. Surya local	Chlorophyll content	Chlorophyll content in the control was 10.72 mg/g, the chlorophyll content in the maize leaves treated with the biostimulant (concentration 0.01%–0.16%) was in the range of 16.43 to 25.88 mg/g on average [54]
Ascophyllum nodosum Seaweed Extract	1.5 kg/ha	"Sangiovese" grapes	Phenolic content	Phenolic content increased by 1.063 mg/cm^2 compared to the control [55]
A. nodosum Seaweed Extract	3kg/ha	"Sangiovese" grapes	Phenolic content	Phenolic content increased by 0.951 mg/cm^2 compared to the control [55]
Biostimulant (chicken feathers) with Fertilizer (300 kg N/ha + 120 kg K/ha)	3.6 L/ha	Maize (*Zea mays* L. cv PR32W86 Pioneer)	Nitrogen content	Nitrogen content of corn leaves increased by 14.4% (first season) 15% (second season) compared to the control [33]
Biostimulant (chicken feathers) with fertilizer (300 kg N/HA + 120 kg K/HA)	7.2 L/ha	Maize (*Zea mays* L. cv PR32W86 Pioneer)	Nitrogen content	Increase nitrogen content by 39.1% (first season) and 33.3% (second season) compared to the control [33]
Protein hydrolysate	5.0 and 2.5 mL/L	Tomato (*Solanum lycopersicum* L.)	Content of lycopene	Increased by 34.9% and 18.0%, respectively, compared to the control [3]
Protein hydrolysate	2.5 mL/L	Tomato (*Solanum lycopersicum* L.)	Ascorbic acid	Increase ascorbic acid content by 27.3% compared to the control [3]
3% corn seed extract	Soaking and praying 1 mM mg plants	Sunflower seed (*Helianthus annuus* L.)	Enzymatic activity of superoxide dismutase, catalase, and peroxidase	Enzymatic activity of superoxide dismutase, catalase and peroxidase increased by 65.5%, 77.8%, and 84.6%, respectively, as compared to the controls [56]

In conclusione:

- ✓ Esistono diversi prodotti ad azione biostimolante in commercio
- ✓ Per alcuni di essi esistono molti studi che ne hanno confermato le proprietà e i meccanismi d'azione
- ✓ Spesso però gli studi sono condotti in condizioni controllate e/o idroponica, in condizioni di campo le condizioni pedoclimatiche possono influenzare gli outcomes
- ✓ Esistono dei target metabolici e fisiologici ricorrenti dei biostimolanti. Tuttavia non è sempre possibile fare delle generalizzazioni
- ✓ Diversi fattori influenzano l'efficacia dei biostimolanti
- ✓ Ruolo importante dei biostimolanti nei sistemi produttivi biologici

GRAZIE PER LA CORTESE ATTENZIONE

Inhortosanitas

Gruppo di ricerca in Orticoltura e Piante Officinali






Sede di Grugliasco
Il Dipartimento di Scienze Agrarie, Forestali ed Alimentari è in Largo Paolo Braccini 2, Grugliasco, TO (10095) - Italia.