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ENHANCING SUGAR BEET'S EARLY GROWTH AND ESTABLISHMENT BY USING PROTEIN-BASED BIOSTIMULANTS

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Enhancing sugar beet's early growth and establishment by using protein-based biostimulants

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Cover pictures: Naked (black), pelleted (brown) and coated (blue) sugar beet seeds (top left) and sugar beet plants under field condition (top right). Sugar beet plant with root (bottom)

Cover pictures by: Dr. Tobias Ekblad (Maribo Hilleshög Research AB, Landskrona, Sweden) and Dr. Ali Hafeez Malik (Nelson Seed Development, Lund, Sweden)

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Keywords: Sugar beet, seed enhancement techniques, biostimulants, protein hydrolysates, amino acids

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Abstract

Sugar beet is the only crop after sugar cane that produces sugar for human consumption and industrial usage. Sugar beet is adaptable to temperate climates between latitudes 30 and 60^{o}N. This crop has been around for more than 2 centuries and several efforts have been made to improve its quality (in terms of sugar content) and vigour (seed germination, emergence and seedling establishment). Until now, sugar beet germination capacity has not been fully explored, a problem that has been linked with the presence of germination-inhibitors present on the pericarp. Sugar beet seeds require sophisticated seed enhancement techniques in order to improve their germination capacity, early growth and establishment. Some of the processes involved with sugar beet seed enhancement techniques include polishing, priming, pelleting and coating among others. Seed pelleting involves the addition of some substances to sugar beet seeds in order improve its shape and size for precision planting. Most of these additives are inorganic chemicals, which are dangerous to our environment. To reduce the effects of these inorganic additives on the environment, this paper considers the use of bio-based products (protein-based biostimulants) in seed enhancement techniques. The aim of this paper is to explore the available information on sugar beet seed enhancement techniques, biostimulants, and their impact on germination, emergence, early growth and establishment.

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1.0 Introduction

Table sugar (sucrose), a white crystalline substance, is a component of the human diet because of its sweetening, energy-giving and preserving properties (Colonna and Samaraweera, 2000; Colonna *et al.,* 2006). Sugar has been available for human consumption over the past two centuries and the average annual consumption per person is about 23 kg (Biancardi *et al.,* 2010). Sugar is produced globally in over 130 countries from either sugar cane or sugar beet and the two crops contribute 75 and 25% of the sugar produced in the world, respectively (Cooke and Scott, 1993; Zimmermann and Zeddies, 2002; Gurel *et al.,* 2008; Biancardi *et al.,* 2010). The average yearly global production of sugar is around 174 million metric tonnes (MT) for the last 10 years (Figure 1) out of which only about 50 million MT is available for international trade (Biancardi *et al.,* 2010; Řezbová, *et al.,* 2013).

Sugar beet (*Beta vulgaris* L.) is the most important sucrose-producing crop in the temperate regions of the world (Rady and Ali, 1999; OECD, 2015). Most of the sugar beet is grown between latitudes 30 and 60^{o}N (Mahmoodi *et al.,* 2008), as a summer or winter crop depending on the climate (Draycott, 1972). The crop is produced in more than 60 countries and provides globally more than 35 million MT of sugar per year (Draycott, 2005; Biancardi *et al.,* 2010; Řezbová *et al.,* 2013). The top ten sugar beet producing countries include Russia, France, U.S., Germany, Ukraine, Turkey, Poland, China, U.K. and Egypt (Biancardi *et al.,* 2010; Řezbová *et al.,* 2013) (Figure 2). Apart from its huge supply of sugar for human consumption, sugar beet has an outstanding ability for liquid biofuel production, giving a range of 100-120 l/t of bioethanol (Leroudier, 2002; Mahmoodi *et al.,* 2008; Panella, 2010; Abts *et al.,* 2013; Dohm *et al.,* 2013; OECD, 2013).

Successful sugar beet cultivation is highly dependent on the supply of high quality seed (Kockelmann and Meyer, 2006). Seed quality is defined by a range of seed characteristics, which include; varietal/genetic and analytical purity, seed germination capacity,uniformity as well as seed health and vigour (McDonald, 1998; Boelt *et al.,* 2018). Good seed quality results in high germination capacity, uniformity of field emergence and good vigour of sugar beet seedlings (Boelt *et al.,* 2018). The above mentioned seed qualities have significant impact on the final yield of sugar beet, both in quantity (root yield) and quality (sugar yield) (Sliwinska and Jendrzejczak, 2002; Reyes *et al.,* 2003; Ashraf and Foolad, 2005; Biancardi *et al.,* 2010; Catusse *et al.,* 2011; Kockelmann and Meyer, 2006; Abts *et al.,* 2013; Vijaya-Geetha *et al.,* 2014; Huang *et al.,* 2016). Sugar beet is particularly sensitive to poor seed quality due to the common practice of precision sowing (drilling) to final stand density, i.e. assuming that close to 100% of the seeds germinate and produce plants (Ashraf and Foolad, 2005). Slow field emergence and establishment of sugar beet can still be a problem despite several improvements that have been made to the seed preparation process, including seed enhancement techniques (priming, pelleting, encrustation and coating) (Kockelmann and Meyer, 2006).

Seed enhancement techniques have led to uniform germination, increased germination speed, and allow the incorporation of active ingredients to protect the seeds and seedling from insects and diseases (Sliwinska and Jendrzejczak, 2002). Seeds enhancement also allows for the application of additional compounds on seed such as fertilizers and biostimulants in order to enhance seedling growth, establishment and ultimately yield and quality (Calvo *et al.,* 2014). Biostimulants have been found to improve crops tolerance to drought and pathogens as well as

improve nutrient uptake and water use efficiency (Vessey, 2003; Berg, 2009; Nardi *et al.* 2009; Asli and Neumann, 2010; Trevisan *et a*l. 2010; Calvo *et al.,* 2014).

This introductory paper is aimed at in-depth exploration of literature about what has been done so far to enhance sugar beet germination, emergence, early growth and establishment by using biostimulants. The second aim was to explore how biostimulants can impact early growth and establishment of sugar beet seedling both in terms of shoot and root development.

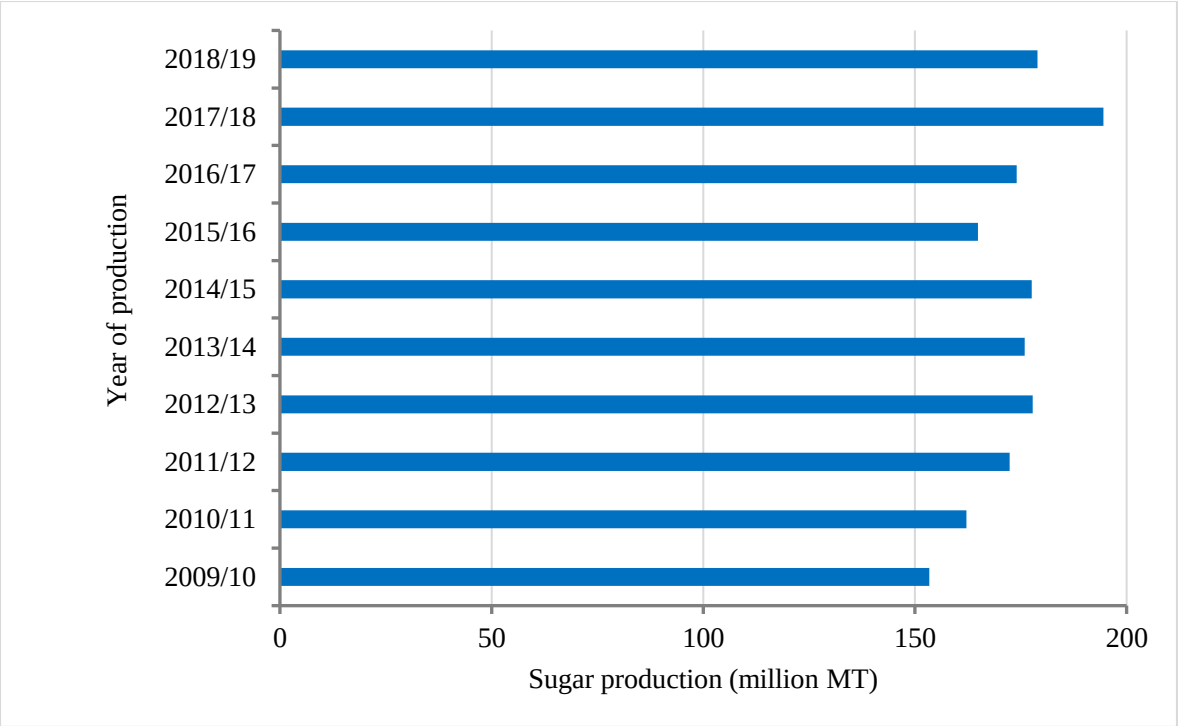
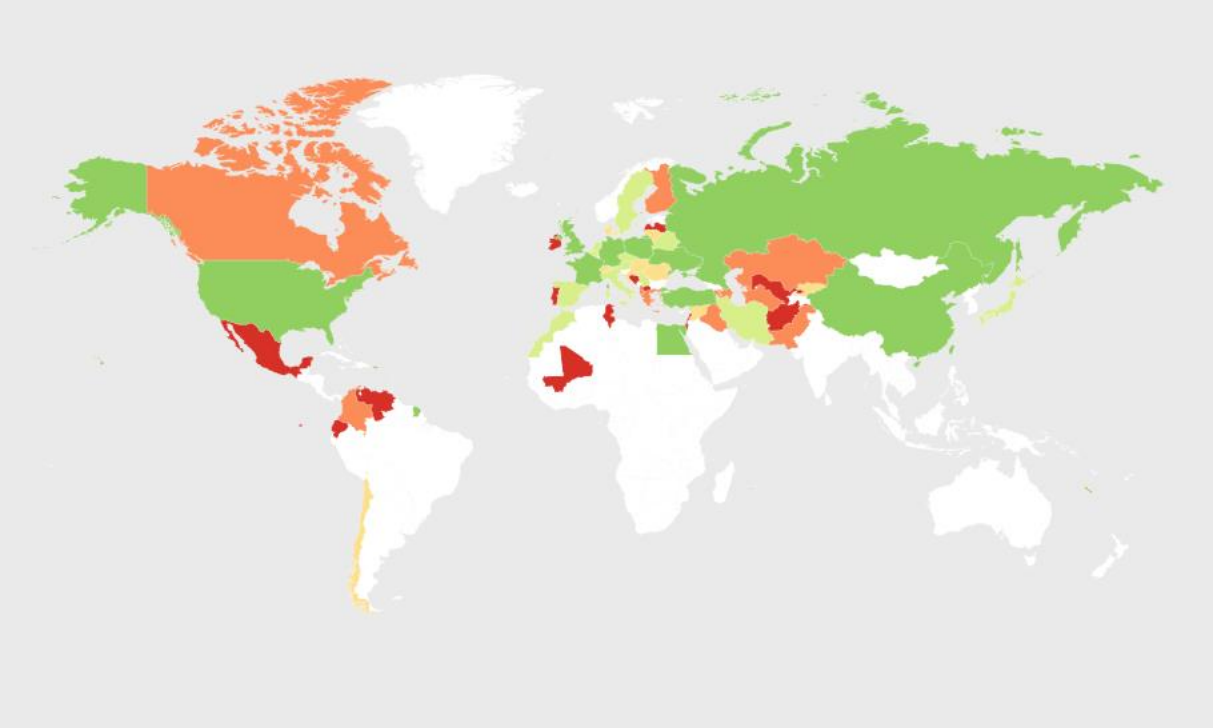


Figure 1: Global sugar production from 2009/2010 to 2018/2019 growing season (Statista, 2019).



Legend

Location	Production statistics (tons)
	0-24,000
	24,000-530,000
	530,000-1,700,000
	1,700,000-5,600,000
	5,600,000-52,000,000

www.knoema.com

Figure 2: Global production statistics for sugar beet. With the green area being the world highest producers including Russia, France, U.S., Germany, Ukraine, Turkey, Poland, China, U.K. and Egypt.

2.0 History and production of sugar beet

2.1 History of beets

Sugar beet was grown as a garden vegetable more than 2000 years ago in Greece, Rome and Mesopotamia (Ford-Lloyd and Williams, 1975; De-Bock, 1986; Zohary and Hopf, 1974; Draycott, 2005; Biancardi *et al.,* 2010; Dohm *et al.,* 2013). *Beta maritima* (sea beet) is the wild ancestor or the progenitor of today's sugar beet plant (OECD, 2006). By the end of the fifteenth century, beet was probably grown all over Europe (Deerr, 1950; Draycott, 2005). Red and yellow beets became popular as salad vegetables during sixteenth and seventeenth centuries (OECD, 2006). Beet was grown as field crop for the first time in the seventeenth century in Spain, although only as fodder for cattle (Cumo, 2013). Andreas Margraff (German chemist) in 1747 discovered the protocol for the extraction of sucrose from sugar beet. Franz Karl Achard (German Breeder and student of Margraff) in 1787 selected sugar beet variety with sucrose content of about 6% fresh root weight by evaluating 23 local sugar beet varieties (Draycott, 2005; OECD, 2006).

2.2 Environmental conditions for sugar beet cultivation

The current root yield of sugar beet (>100 t/ha) has been attributed to a combination of a breeding progress (selection for high yielding varieties) and optimal weather conditions in Europe (Hoffmann, 2017).

Sugar beet is a sun-loving crop; it does not require too much rain and cloud (Finch *et al.,* 2002). In Europe sugar beet is sown early during the spring in order to provide closed canopy for longer period of sunshine interception (Petkeviciene, 2002; Hoffmann and Kluge-Severin, 2011; Kirchhoff *et al.,* 2012). Root yield loss of 300 kg ha^{-1} and 50 kg ha^{-1} of white sugar yield has been reported when sowing is delayed by average of one day (Petkeviciene, 2009). Sugar beet sowing time also depends on the cultivation technology and it is influenced by soil moisture (Romaneckas and Sarauskis, 2003; Petkeviciene, 2009). Fast sugar beet emergence is obtained when the soil moisture in the seedbed is 20–23%, and air and soil temperature ranges between 15–25°C (Copeland and McDonald, 2001; Hoffmann, 2017).

Most beets are grown on calcareous soils with a clay content between 10-25% and a high fertility level with neutral acidity (pH 7). Nitrogen is important for the sugar beet crop as it stimulates foliage canopy towards adequate solar interception (approximately 90% of the solar radiation) as soon after sowing as possible (Draycott and Christenson, 2003; Jaggard *et al.,* 2009). However, nitrogen is not important at harvest, as it limits sugar extraction (Draycott and Christenson, 2003). Ideally, lime should be applied to the soil one year ahead of planned sugar beet cultivation. Fertile and deep soils are often reserved for sugar beet cultivation due to the high economic return (Olsson and Olsson, 2004; FAO, 2009). Days to maturity of sugar beet from the day of sowing range from 120-250 days depending on the region of cultivation and month of the year of planting (Table 1).

2.3 Economic importance of sugar and sugar beet

Sugar is politically and economically high-profile commodity for the major sugar producing countries in the world (AB Sugar, 2019). Globally, sugar is traded in both raw (brown sugar) and refined (white sugar) forms representing 55 and 45% of the international trade respectively (AB Sugar, 2019). The global sugar production is dominated by Brazil, India and the European Union (EU) (OECD/FAO, 2015) (Figure 3). The EU is the world's leading producer of beet sugar with an average annual production of 17.7 million MT (OECD/FAO, 2015). The EU sugar reform between 2013 and 2017 has led to reduced production and exports as well as increased imports (OECD/FAO, 2010) (Figure 4).

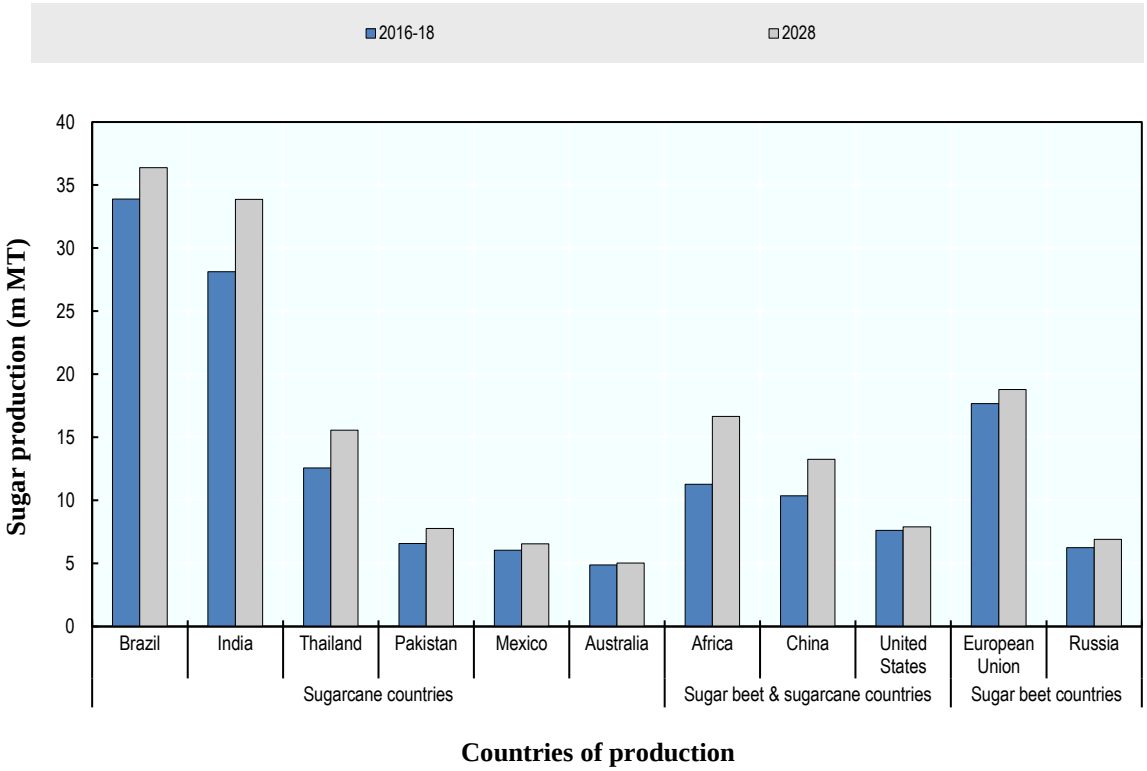


Figure 3: Sugar production in major producing countries classified by crop (OECD/FAO, 2015)

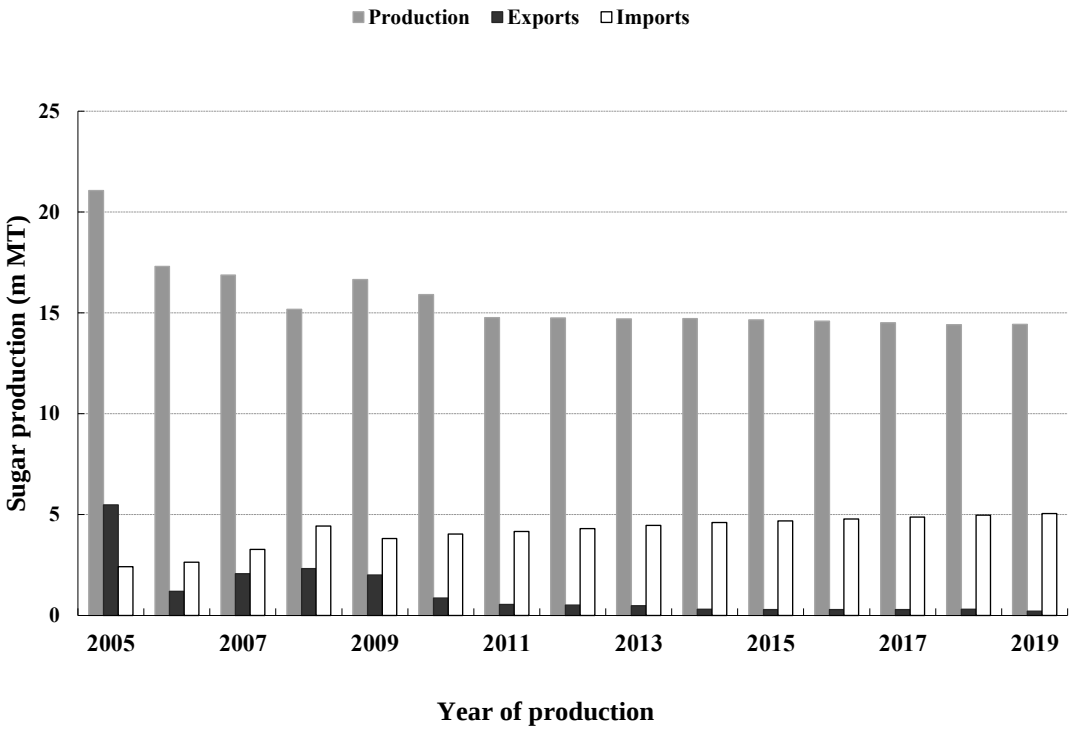


Figure 4: EU sugar reforms lead to lower quota production, fixed exports and rising imports (OECD/FAO, 2010).

Table 1: Developmental stages (days) of sugar beet under different climate in production regions.

Region	Sowing period	Sowing-emergence	Sowing-maximum canopy cover	Sowing-start canopy senescence	Sowing-Maturity
Mediterranean	Nov	10-14	100-130	130-160	200-250
	Feb	16-20	80-100	100-130	150-180
Northern Europe	Mar	18-22	80-100	100-120	160-190
	Apr	18-22	70-90	90-120	150-180
India	Oct	8-12	40-60	60-80	120-140
USA	Sept	8-20	90-110	110-130	240-270
	Mar	16-20	80-100	100-120	170-190
	Jun	7-17	70-80	80-90	130-150

Rinaldi, 2012.

3.0 Botany of sugar beet

3.1 Sugar beet plant morphology

Sugar beet is a typical vegetative crop with a biennial life cycle, belonging to the family *Amaranthaceae* (Getz, 2000; Hermann *et al.,* 2007). After sowing in spring, it produces leaf and root mass until harvest in autumn. Sugar beet leaf are ovate or cordate in structure depending on the varieties (Højland and Pedersen, 1994; OECD, 2006). As a result of secondary thickening, the storage root is made up of 15–20% sucrose contents (FAO 2009; OECD, 2015). Sugar beet enters its reproductive phase only after exposure to a long period of cold temperatures (<4°C) in the second year, which results in shoot elongation (bolting) and flowers are produced (Elliot and Weston, 1993; OECD, 2006). Each flower contains two stigmas (female parts), and the seeds are in clusters and enveloped in a woody covering (calyces) (OECD, 2006).

3.2 Sugar beet seed morphology

Sugar beet seeds are small irregular star-shaped of about 10 mg per seed in weight (OECD, 2006). The sugar beet seed is classified as a fruit, which is referred to as achene (OECD, 2006; Hermann *et al.,* 2007). Achene is a "small, dry, indehiscent fruit, with their seeds not adhering to the carpel and do not split open when ripe" (Hermann *et al.,* 2007; Marzinek *et al.,* 2008). The sugar beet fruit consists of a fruit cavity and a pericarp which is the outermost layer covering the operculum or fruit cap (Orzeszko and Podlaski, 2003; Hermann *et al.,* 2007; Abts *et al.,* 2013) (Figure 4).

3.3 Sugar beet seed germination physiology

Seeds germination in sugar beet like every other crop, is largely controlled by temperature and availability of water if oxygen is present (Gummerson 1986; Sadeghian and Yavari, 2004). Sugar beet seed do not germinate evenly, and this uneven germination is mostly related to some inhibitory substances reportedly found on the seed pericarp (Tolman and Stout, 1940; Battle and Whittington, 1969). These inhibitory substances have been identified and they include: free ammonia from the pericarp (Stout and Tolman, 1941); osmolytes in the seed coat (Duym *et al.,* 1947); and unsaturated yellow oil. Unsaturated yellow oil has been reported in other crops and can be removed by prolonged washing (De-Kock and Hunter, 1950). Another germination–inhibitor of sugar beet is the presence of salt crystals on the thick-wall sclereids of the pericarp, which form an osmotic solution with low water potential in the presence of water (Orzeszko-Rywka and Podlaski, 2003; Hermann *et al.,* 2007).

Sugar beet seeds germination is improved by the removal of the soft outer part of the pericarp, through the process called polishing. The aim of polishing is to reduce the irregular shape/size of the seed to an optimal grade suitable for pelleting (Kockelmann and Meyer, 2006). However, during the process of polishing, germination-inhibitors that are present on the seed pericarp are also removed thereby allowing improved germination of the sugar beet seed (Kockelmann and Meyer, 2006; Abts *et al.,* 2013). Nevertheless, polishing must be done gently to avoid cracks in the pericarp and embryo damage, especially to the radicle. The pericarp also serve as

physical barrier for water and oxygen uptake in addition to the presence of germination-inhibitors, thereby retarding germination (Abts *et al.*, 2013).

4.0 Seed enhancement techniques

4.1 Seed treatments

Seed treatments is defined by the International Seed Federation (ISF), as "the application of biological, physical and chemical agents to seed that provide protection to seeds and plants and improve the establishment of healthy crops" (Sharma *et al.,* 2015). A number of seed treatment methods have evolved over the years ranging from physical seed treatment (PT) (hot water treatment, dry heat treatment, aerated heat treatment and radiation treatment) to chemical and biological seed treatment (CBT). The CBT includes different seed immersion techniques, as well as seed priming, seed pelleting and seed coating (Sharma *et al.,* 2015). Seed treatments have been used on many crop seeds for a variety of purposes including alleviation of stresses associated with soil environment (biotic or abiotic) and improving plant growth. For instance, Avelar *et al.,* (2012) reported treatment with systemic chemicals to control plant pathogens within the plant system. Seed treatment with beneficial microorganisms, which help to fix Nitrogen and enhance uptake of nutrients, is also possible. Another example is seed physical treatments to control seed-borne pathogens and seed coatings or pelleting to improve seed shape for planting or provide other benefits (Avelar *et al.,* 2012). Taylor and Harman, (1990) also cited examples of physiological seed treatments that enhance germination rate and plant performance. There are treatments that affect seed moisture relationships and result in improved seed storability or performance (Taylor and Harman, 1990).

4.1.1 Seed priming

Sugar beet seeds are frequently subjected to priming as a pre-treatment exercise. Priming contributes to an improvement of germination characteristics, especially to speed and uniformity of emergence under stressful conditions (Kockelmann and Meyer, 2006). The seed priming technique includes a partial seed hydration to initiate germination-metabolic processes without actual germination thereafter a re-drying of the seed to close to their original weight to permit routine handling (Mahmood *et al.,* 2016). A range of compounds have been used in priming of different crops species, namely; osmotic compounds (polyethyleneglycol- PEG), water (hydropriming) and various biological compounds (biopriming) (Kockelmann and Meyer, 2006; Mahmood *et al.,* 2016).

4.1.2 Seed pelleting

Seed pelleting was basically developed to increase the apparent seed size and weight and to alter seed shape for precision seed planting (Taylor *et al.,* 2001; Sharma *et al.,* 2015). Seed pelleting is the most sophisticated seed treatment technique (Sharma *et al.,* 2015). Many crops have small and irregular shaped-seeds, which does not permit accurate metering by mechanical planting equipment (Taylor and Harman, 1990). Seed pelleting therefore has the following advantages; (i) drilling to final stand, (ii) homogeneity of drilling, (iii) application of active ingredients (including biostimulants) without the risk of phytotoxicity (Kockelmann and Meyer, 2006). Sugar beet seed as well as vegetable and flower seed companies have developed and employed pelleting technique on a commercial scale (Taylor *et al.,* 1997). The focus of pelleting is to allow only one seed per pellet and to prevent seeds from sticking to one another.

The pellet general include, the seed mass wrapped up within two components, a binder (or adhesive) and an inert filler thereby increasing the seed weight by 100-5000% of the original seeds weight (Taylor *et al.,* 1997). Freshly produced seed pellets are usually wet since water is required to hydrate the binder, and therefore the pelleted seeds must be dried to desired moisture content before storage (Taylor *et al.,* 1997). Taylor and Harman (1990), have reviewed the use of various binders used for seed pelleting and described the use of gum arabic, gelatin, starch, methyl-cellulose, polyvinyl alcohol, polyoxyethylene glycol-based waxes, and carboxymethyl cellulose. Fillers or particulate matters used for pelleting include calcium carbonate, limestone, gypsum, talc, vermiculite, diatomaceous earth, kaolin clay, bentonite, zeolite and peat.

Sachs *et al.,* (1981) and Sachs *et al.,* (1982) have reported that clay and sand pellets acted as physical barrier to water and oxygen diffusion and mechanical barrier to radicle protrusion of sweet pepper seeds. Durrant and Loads, (1986) also found out that clay pellet applied to sugar beet seeds reduced emergence when sown in wet soil conditions, whereas more porous pelleting materials gave 5-10% greater stand than the clay pellet. Therefore, the composition of the pellet can have a direct influence on germination, especially under adverse soil conditions. In order to escape the adverse effect of soil moisture on germination of pelleted seeds, moist pellets have also been developed. Seeds treated as moist-pellet (quick pill) should be stored at 4^{o}C and must be sown within 14 days (Taylor and Harman, 1990). Moist pelleting system is necessary for biological organisms that are desiccation-intolerant or that need high moisture levels for optimum performance (Taylor and Harman, 1990).

4.1.3 Seed coating

Seed coating includes any process for the direct application of a material to seeds without changing its general size or shape (Taylor *et al.,* 1998, Taylor *et al.,* 2001; Avelar *et al.,* 2012). Active ingredients both in the form of dry powders and as slurry have been applied to seeds to form coatings around the seeds. However, dry powder materials do not generally adhere well to the seed surface thereby resulting in poor loading, lack of uniformity, and dust problems (Khan *et al.,* 1980). Application of slurries instead often improves the uniformity and helps in overcoming other problems associated with dry powder application. Slurry treatments include adhesives (stickers, glues, or binders) to improve retention of materials applied to seeds. Adhesives used for this application include methyl-cellulose, dextran, gum arabic, vegetable or paraffin oils and a range of synthetic binders (Halmer, 1988; Sharma *et al.,* 2015).

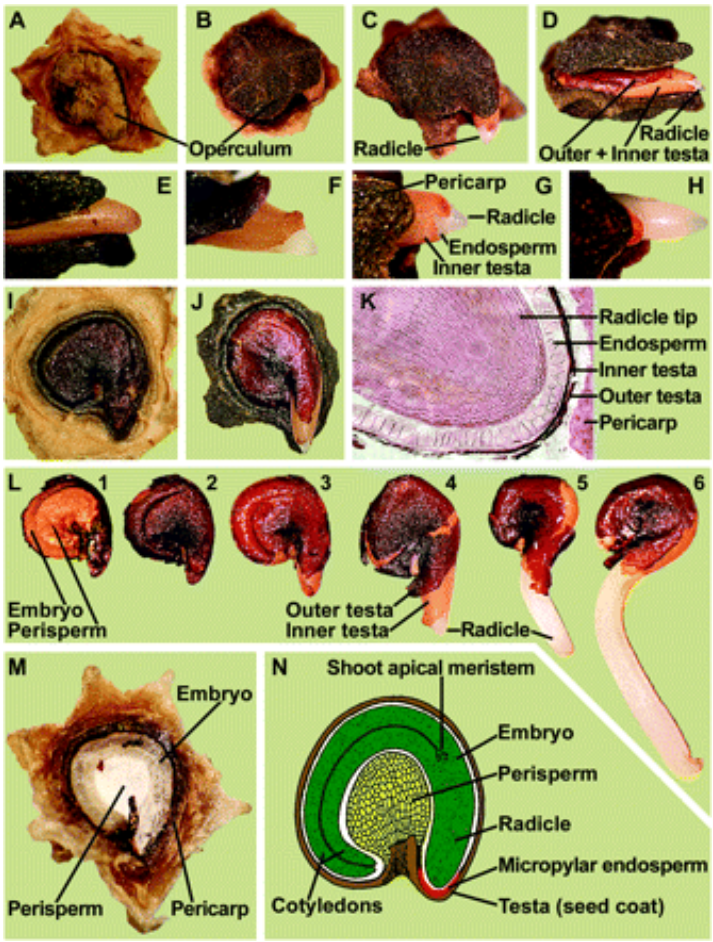


Figure 5: Structure of mature fruits and seeds of *Beta vulgaris*. (A–H) Visible events during the incubation of sugar beet fruits in water: (A) Dry fruit. (B, E) Operculum opening; note that the radicle tip is still enclosed by the micropylar endosperm and the inner testa. (C, D, F–H) Radicle emergence through the seed covering layers (testa and endosperm) is the completion of germination. (I, J) Seed germination studied with deoperculated fruits. The sugar beet seed has a lentil-like structure (about 3 mm diameter and 1.5 mm thick) and occupies a horizontal position within the fruit. (J) Radicle emergence through the seed covering layers (testa and endosperm) is the completion of germination. (K) Microscopic section through a dry fruit showing the radicle tip enclosed by the covering layers. (L) Distinct stages of sugar beet seed germination: isolated dry seed (1, 2); note that the testa was removed in (1) to make the embryo and perisperm visible. Imbibed seed showing rupture of the outer testa (3) and radicle protrusion through all the seed covering layers (4–6). (M) Section through a mature sugar beet fruit. The curved embryo completely encloses the perisperm, which is dead starch storage tissue localized in the seed centre. (N) Drawing of a sugar beet seed; modified from Bennett and Esau (1936) and reproduced by the kind permission of the United States Department of Agriculture. Based on the peripheral location of the embryo, the sugar beet seed can be structurally classified as being perispermic and P-type (Hermann *et al.*, 2007).

5.0 Biostimulants

5.1 Agriculture and Biostimulants

The use of chemical fertilizers and pesticides, a result of the green revolution in the 1960s, have partly contributed to the environmental pollution we see today (Canellas *et al.,* 2015). Both minerals and chemical compounds can be washed off the field or drained into water bodies or ground water resources, thereby polluting air and water (Halpern *et al.,* 2015). Also, the industrial production of these compounds is energy-intensive, thereby contributing to global greenhouse gas emissions. Modern agriculture is aimed at reducing inputs without reducing the yield and quality in an organic, sustainable or environmental friendly systems (Bulgari *et al.,* 2015). This goal is achievable by breeding programs it will however be species-specific and time-consuming.

Developing crops with robust root systems and higher nutrient-uptake efficiency may combat the above challenge (Halpern *et al.,* 2015). Such crops can be enhanced by the application of biostimulants to crop leaves, seeds, or soil in order to stimulate general plant growth and development (Canellas *et al.,* 2002; Khan *et al.,* 2009; Zandonadi *et al.,* 2007), activates several physiological processes that enhance efficient nutrients uptake (Pinton *et al.,* 1999), beneficial microbial populations and allowing the reduction of fertilizers consumption (Chen, 2006; Vessey, 2003). All these activities in turn lead to increased crop yield (Kunicki *et al.,* 2010).

Biostimulants is defined as "substances or materials, with the exception of nutrients and pesticides, which, when applied to plants, seeds, or growing substrates in specific formulations, have the capacity to modify physiological processes in plants in a way that provides potential benefits to growth, development, or stress response" (Halpern *et al.,* 2015; Wilson *et al.,* 2018). Protein hydrolysates and other plants and animal products as well as some beneficial microorganisms have been found to enhance plant growth (Rouphael and Colla, 2018). These organic substances are termed biostimulants. Other N-containing compounds, humic and fulvic acids, botanicals, chitosan and other biopolymers are also included in this category (Tanou *et al.,* 2017). Biostimulants are normally used in the seed industry as post-harvest treatment on seed prior to sowing (Wilson *et al.,* 2018), through seed enhancements techniques.

The market of biostimulants has been rapidly growing since the last 10 years (Rouphael and Colla, 2018), and it is currently at 2 billion USD with an estimated 50% increase by 2021 (Rouphael and Colla, 2018).

5.2 Benefits of biostimulants

The beneficial effects of biostimulants has been attributed to auxins and gibberellin metabolism, enhanced nitrogen uptake as well as reactive-oxygen/nitrogen species and hormonal signaling (Tanou *et al.,* (2017). Biostimulants are believed to be interacting with plant signaling processes thereby improving plant tolerance to stresses and so improving plant productivity (Brown and Saa, 2015). Some of the effects of biostimulants include; positive changes in soil structure or nutrient solubility, root morphology, plant physiology, and symbiotic relationships (Nardi *et al.,* 2016). One of the major challenges of the use of biostimulants is the determination of their function, which is due to the variations in the sources of the materials, which is as a result of different industrial manufacturing processes.

5.3 Classification of Biostimulants

Biostimulants are categorized into four major groups namely:

a. humic substances (HS),
b. protein-based biostimulants
    i. protein hydrolysates (PHs) and
    ii. amino acid formulations (AA),
c. seaweed extract (SE), and
d. plant-growth-promoting microorganisms.

5.3.1 Humic substances (HS)

Humic substances (HS) are byproducts of microbial metabolism of dead organic matter that are formed in the soil (Nardi *et al.,* 2016; Canellas *et al.,* 2015). HS are very common and make up 60% of the organic matter in the global soils (Muscolo *et al.,* 2007). HS are made up of many small organic molecules that are held together by hydrophobic interactions and hydrogen bonds (Sutton and Sposito, 2005; Halpern *et al.,* 2015). The sources of HS include peat, soil, manure compost, green waste compost, brown coal and earthworm casts (Rose *et al.,* 2014; Halpern *et al.,* 2015). HS sustain plant growth and terrestrial life in general. Their functions include; regulation of both soil carbon and nitrogen cycling, growth of plants and microorganisms, the fate and transport of anthropogenic-derived compounds and heavy metals, and the stabilization of soil structure (Piccolo, 1996; Canellas *et al.,* 2015). HS can be applied to crops foliage, or through irrigation water as well as direct application to soil (Salman *et al.,* 2007; Yildirim, 2007; Katkat *et al.,* 2009).

Rose *et al.,* (2014) have reported that exogenous application of HS increased shoot and root dry weights of different plant species by about 22%. The most critical factors regulating the effect of HS on plant growth and physiology are application rate, sources of HS and plant type (Canellas and Olivares, 2014). Canellas *et al.,* (2015) reported that monocot respond to exogenously applied HS better than dicots, although the reason is unclear.

5.3.2 Protein-based biostimulants (PBB)

The use of protein-based biostimulants to improve crop growth and physiology have been reviewed (Nardi *et al.,* 2016). PBBs are hydrolyzed products of protein-based wastes usually from Agro-allied (animal or crop) industries (Schiavon *et al.,* 2008).

Protein-based biostimulants are either protein hydrolysates (PHs) consisting of mixture of different categories of peptides and individual essential and non-essential amino acids from animal/plant origin (Calvo *et al.,* 2014; Colla *et al.,* 2014; Nardi *et al.,* 2016).

5.3.2.1 Protein hydrolysates (PHs)

Protein hydrolysates are derived mainly from chemical and/or enzymatic hydrolysis of proteins of crops (seeds, hay) and animals (leather, viscera, feathers, blood) origin (Maini, 2006, Schiavon *et al.,* 2008). There are reports of PHs stimulating root and leaf biomass when applied to crops (Zhang *et al.,* 2003; Ertani *et al.,* 2009; Cristiano *et al.,* 2018). PHs applied to plant foliage or roots have been effective to increase Fe and N metabolism, nutrient uptake, water and nutrient use efficiencies for both macro and microelements (Cerdán *et al.,* 2009; Ertani *et al.,* 2009; Halpern *et al.,* 2015). Improved nutrients uptake in PH-treated plants

have been attributed to; (i) increased soil microbial activity and soil enzymatic activities, (ii) improved micronutrient (Fe, Zn, Mn and Cu) mobility and solubility, (iii) modifications in the root architecture (root length, density and number of lateral roots) of plants and (iv) increase in nitrate reductase, glutamine synthetase and Fe (III)-chelate reductase activities (Colla *et al.,* 2014). PHs contained specific peptides and amino acids (e.g. tryptophan- precursors of phytohormone biosynthesis), which help to influence plant development (Colla *et al.,* 2014).
PHs widely varied in their protein/peptides and free amino acid contents, ranging from 1 to 85% (w/w) and 2–18% (w/w), respectively (Calvo *et al.,* 2014). Animal-derived PHs usually contain a higher amount of total amino acids than plant-derived PHs (Ertani *et al.,* 2009). Plant-derived PHs contain other compounds that can contribute to the biostimulant action in addition to amino acids and peptides. These compounds include fats, carbohydrates, phenols, mineral elements, phytohormones and other organic compounds (e.g., polyamines).
Corte *et al.,* (2014) has debunked the fear around the safety of animal-protein hydrolysates. They confirmed that chemically or enzymatically hydrolysed animal protein showed no toxic effects on the ecosystems (Calvo *et al.,* 2014).

5.3.2.2 Amino Acids (AA)

Amino Acids (AA) are organic compounds containing an amine functional group and a carboxylic acid functional group (Huang *et al.,* 2011). In addition to the 20 common amino acids used for protein biosynthesis, there are 250 more AA (non-protein amino acids) that are known to be involved in different other plant functions (Huang *et al.,* 2011; Vranova *et al.,* 2011; Calvo *et al.,* 2014). The functions include tolerance to stresses (biotic and abiotic), signaling, N storage, and chelation of metals as phytosiderophores (Huang *et al.,* 2011; Vranova *et al.,* 2011). AA can be absorbed directly by plant roots into the xylem (Biernath *et al.,* 2008), through specific transporters in the roots (Nasholm *et al.,* 2009) or through diffusion into the leaves (Kolomaznik *et al.,* 2012; Pecha *et al.,* 2012). Halpern *et al.,* (2015) reviewed the application of AA on crops and its effect on the morphology and physiology of crops which include; increased biomass production (Shehata *et al.,* 2011), tolerance to biotic and abiotic stresses (Cohen and Gisi, 1994; Maini, 2006; Polo *et al.,* 2006) and increase the antioxidant content of the leaves (Ardebili *et al.,* 2012). The mechanisms by which AA improves soil processes is similar to PHs (Halpern *et al.,* 2015).

5.3.2.3 Plant physiology and metabolism of PHs and AA

PHs have been reported to increase nitrogen assimilation by stimulating carbon and nitrogen metabolism (Calvo *et al.,* 2014). It has also been shown that activities of several enzymes, including NAD-dependent glutamate dehydrogenase, nitrate reductase, malate dehydrogenase, isocitrate dehydrogenase, citrate synthase, nitrite reductase, glutamine synthetase, glutamate synthase and aspartate aminotransferase in the tricarboxylic acid (TCA) cycle as well as N reduction and assimilation of maize were enhanced following application of PHs (Maini 2006; Schiavon *et al.,* 2008; Calvo *et al.,* 2014).

Application of glutamate (AA) has been found to promote root growth of *Arabidopsis*. This result suggests the signaling role for glutamate for root growth (Walch-Liu *et al.,* 2006; Forde and Lea, 2007).

5.3.2.4 Plant defenses to biotic and abiotic stress

The application of PHs and specific AA (e.g. proline) has led to improved plant defense mechanisms to abiotic stresses, including salinity, drought, and oxidative conditions (Ashraf and Foolad 2007; Chen and Murata 2008; Calvo *et al.,* 2014). Kauffman *et al.,* (2007) showed that there was increase in photochemical efficiency and cell membrane integrity compared to control in perennial ryegrass with foliar application of PH (animal hydrolysate) exposed to prolonged high air temperature stress. Apone *et al.,* (2010) have reported the expression of three stress marker genes, two of which enhance the tolerance of cucumber plants to oxidative stress when a mixture of AA-peptide-sugar was applied (Apone *et al.,* 2010). PH applied to maize grown in hydroponic condition under salinity stress showed increase in plant biomass and favorable physiological parameters (Ertani *et al.,* 2013).

Beta-aminobutyric acid (BABA) and gamma-aminobutyric acid (GABA) which are non-protein amino acids have been reported to increase plant resistance to abiotic and biotic stresses (Shang *et al.,* 2011; Calvo *et al.,* 2014).

5.3.2.5 Plant tolerance to heavy metals toxicity

The role of PHs and AA to plant tolerance heavy metals toxicity has been reviewed by Calvo et al., (2014). Plants subjected to heavy metal stress and some metal-tolerant plants exhibit increase accumulation of proline (Sharma and Dietz, 2006). Proline acts as osmoregulator in plant exposed to heavy metal stress by counteracting water deficit and by chelating metal ions within plant cells (Calvo et al., 2014). Another AA that is associated with Nickel-hyperaccumulation in plants is histidine (Calvo et al., 2014). Histidine is said to be involved in Ni-transport from root to shoot (Krämer et al., 1996; Kerkeb and Krämer 2003). Other AAs and peptides (e.g. glutamine and glutathione respectively) have also been reported as important chelates of metal ions such as Zn, Ni, Cu, As and Cd (Sharma and Dietz, 2006; Sytar et al., 2013; Calvo et al., 2014).

5.3.4 Seaweed extracts (SE)

Seaweed extracts may be considered the oldest biostimulants, as it has been used for many centuries (Calvo *et al.,* 2014). SE has been applied directly to crops for improved crop productivity or used as soil-compost to promote soil structure and fertility (Khan *et al.,* 2009; Craigie, 2011). Several researchers have established the positive effects of SE as biostimulants in enhancing seed germination and establishment. SE, like other biostimulants also improve plant vegetative and reproductive phases, as well as improve tolerance to biotic and abiotic stresses (Rayorath *et al.,* 2008; Khan *et al.,* 2009; Craigie, 2011; Mattner *et al.,* 2013; Michalak *et al.,* 2015). Other roles of SE to plant health include, heavy metal-chelation and nutrient-use-efficiency (Tarakhovskaya *et al.,* 2007; Calvo *et al.,* (2014).

5.3.5 Plant-growth-promoting Bacteria (PGPB)

PGPB are biological agents, which are as effective as pure chemical in managing abiotic and biotic stresses in plants (Maheshwari, 2011). They help to enhance plant growth (Dardanelli *et al.,* 2009; Figueiredo *et al.* 2010) by direct and/or indirect mechanisms which include; (i) minerals solubilization and availability to plants, (ii) improving plants tolerance to abiotic stresses like drought, salinity and metal toxicity and (iii) tolerance to disease-causing organisms

by producing some metabolites (Glick, 1995; Jha *et al.*, 2013) and consequently increasing crop yield (Figueiredo *et al.* 2010). A large number of PGPB are commonly found in the plant rhizoplane (roots), and/or the plant rhizosphere (around the roots), generally up to 1 mm from the root surface (Dardanelli *et al.,* 2009). The main groups of PGPR belong to the phyla Cyanobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria (Figueiredo *et al.* 2010). The role of specific strains of PGPB and rhizobia in plant-growth promotion include; N-fixation, biofertilizer activities, and biological control (Dardanelli *et al.,* 2009). Rhizospheric bacteria (Azospirillum and Azotobacter) have been used for root induction of micropropagated jojoba (*Simmondsia chinensis*), photinia and ornamental grasses (Carletti *et al.,* 1998; Larraburu *et al.,* 2007; Dardanelli *et al.,* 2009). PGPR therefore has the possibility of replacing all or at least some synthetic plant hormones used in plants *in vitro* cultures (Dardanelli *et al.,* 2009). PGPB inoculants promote plant growth through at least one of the following mechanisms: (i) suppression of plant disease (bioprotectants), (ii) improved nutrients acquisition (biofertilizers) and (iii) phytohormone production (biostimulants) (Figueiredo *et al.,* 2010). Examples of PGPB include; Bacillus, Streptomyces, Pseudomonas, Burkholderia, and Agrobacterium, being the biological control agents predominantly studied and increasingly marketed (Tenuta, 2003).

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