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Stress in grasses:
understanding the physiology and management for mitigation.

MAIS INTEGRIDADE

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Introduction

Over thousands of years of evolution, plants, as sessile organisms, have undergone adjustments to adapt to the different conditions of the environment to which they are exposed. In general, this adaptation comes from the identification of a set of small stimuli, which need to be identified and transcribed by plants to transform into an adaptive response. In superior plants, this transmission is carried out by chemical messengers and/or by endogenous hormones (Taiz et al., 2017).

The definition of a plant hormone is an organic, non-nutrient compound, naturally occurring and produced in the plant, which at low concentrations (10^{-4} M) promotes, inhibits or modifies morphological and physiological processes in the plant (Castro, 1998; Cato, 2006). The literature also reports that for there to be responses in plants, the hormone must be in sufficient amounts in cells, be recognized and captured by specific receptors and have its responses increased by secondary messengers (Taiz & Zeiger, 2013; Taiz et al., 2017). It is worth remembering that plant hormones play important roles ranging from germination stimuli to maturation processes and, therefore, are fundamental in the components of productivity and quality of the harvested product (Cato, 2006).

According to the European Biostimulants Industry Council (EBIC) biostimulants can contain substances or microorganisms that stimulate the natural processes of plants in order to improve or increase the absorption and/or efficiency of nutrients, tolerate stress, improve quality and increase yields of the crops to which they are applied (Schukla et al., 2019; Rosa et al., 2021).

The use of biostimulants and/or biofertilizers in the most diverse crops, such as soy, corn, beans, wheat, barley and vegetables in general, such as: brassicas, tomato, strawberry, grape, among others, has gained space in modern agriculture (Rayorath et al. al., 2008; Kumar & Sahoo, 2011; Schukla et al., 2019; Rosa et al., 2021; Shukla & Prithiviraj, 2021), especially when it comes to mitigating the different types of stress to which plants are subjected. Among biofertilizers, *Ascophyllum nodosum* has been highlighted to promote plant growth and development, with its use gaining prominence from seed germination to resistance to biotic or abiotic stresses (Khan et al., 2009; Kumar & Sohoo, 2011; Carvalho, 2013; Shukla et al., 2019).

Ascophyllum Nodosum and its applications in agriculture

Ascophyllum nodosum (L) Le Jol. is a macroscopic seaweed, rich in polysaccharides and unsaturated fatty acids, with a large amount of enzymes and bioactive peptides (Shukla et al., 2019; Rosa et al., 2021).

It is worth remembering that *A. nodosum* extract has several precursors and hormone regulators in plants, among which cytokinin (Wani et al., 2016; Khan et al., 2011;), gibberellic acid (Wally et al., 2013) and auxin (Rayorath et al., 2008; Shukla et al., 2019). It is worth noting that auxin is a hormone produced in the shoot and is related to root growth and that cytokinin is a hormone synthesized in the roots, redistributed through xylem to the shoot of plants, stimulating their development (Salisbury & Ross, 2012; Taiz et al., 2017).

Due to its versatility in how it is used, *A. nodosum* extract is applied for everything from seed treatment to foliar sprays, localized “drench” applications and fertigation or through a combination of these methods (Mackinnon et al., 2010). As responses to these applications, improvements have been observed in vegetative growth, increased absorption of nutrients, stimulation of the antioxidant system, activation of defense metabolism and attenuation of stresses, mainly water, thermal and saline (Chouliaras et al. 2009; Rosa et al., 2021). In general, all these benefits resulted in improved productivity and also in the quality of the harvested product.

a) Seed Treatment

Using *A. nodosum* in seed treatment increased the activity of an amylase that is independent from gibberellin, thus improving germination potential, embryonic development and maintaining seed vigor (Rayorath et al., 2008). These data corroborate those found by Carvalho (2013) for beans and soy and those found by Santos et al. (2019) for sunflower, where seeds treated with *A. nodosum* extract showed a higher emergence speed rate, root length, plant height, number of pods, number of grains per pod and productivity.

In the same way that it happened for bean, soy and sunflower crops, the application of *A. nodosum* in corn seed treatment promoted root growth (48%), increased the number of grains per ear (62%) and the dry weight of grains (103%) when compared to the control treatment (Carvalho, 2013). This increase in the root system is due to the increase in amylase independent from gibberellin promoted by the application of *A. nodosum* (Rayorath et al., 2008). It is well-known that amylase increases the metabolic conversion that originate the seedlings, favoring the growth and establishment of plants in the field (Marcos Filho, 2015). For the developmental and reproductive parameters, it is related to the increments to the greater mobilization of photoassimilates to the pods and fruits when the plant is at this stage (Khan et al., 2009; Marcos Filho, 2015).

The literature has also shown that corn plants that were treated with *A. nodosum* showed greater root length and volume, especially secondary roots (Figure 1), which are responsible for absorbing water and nutrients (Ertani et al., 2018).

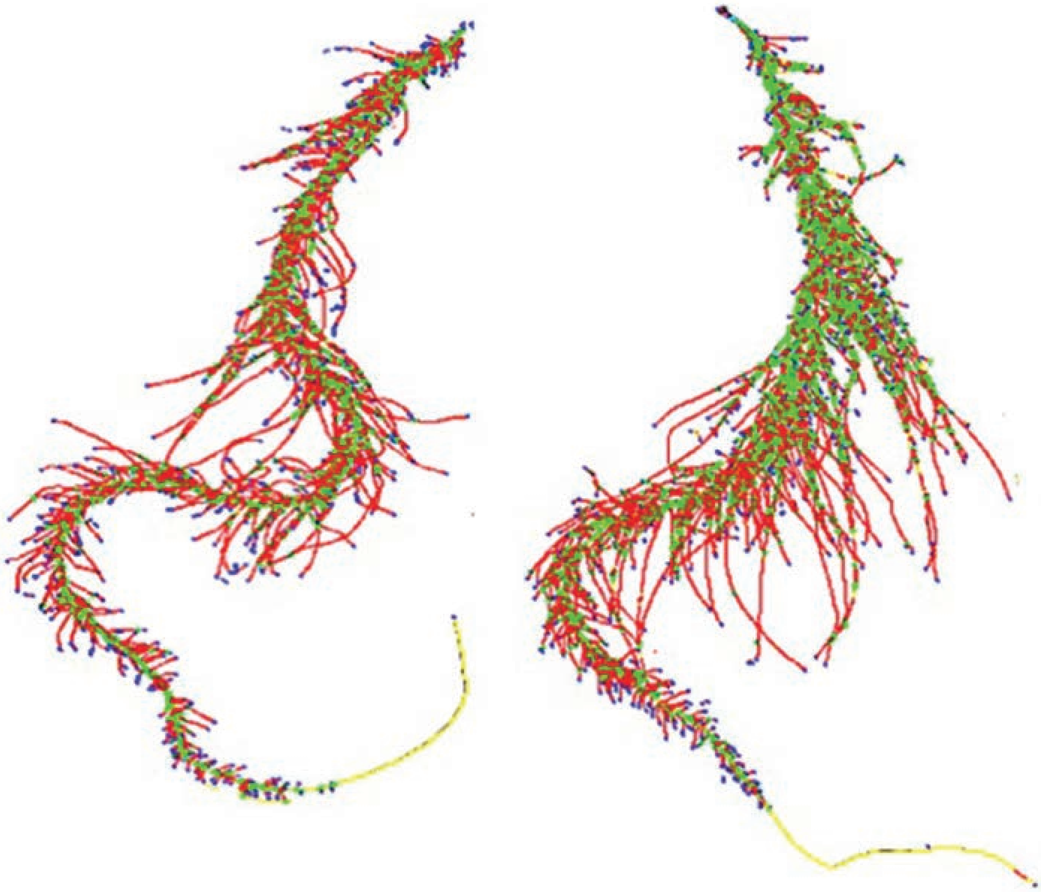


Figure 1. Comparison between untreated corn roots (left) and corn roots treated with algal extract (right). The image was obtained using the Epson Expression 10000XL 1.0 system, equipped with the WinRHIZO analysis system. (Ertani et al., 2018).

b) Efficiency in using nutrients and improvements in the harvested product

Recent studies report that the application of *A. nodosum* improves the absorption and efficiency of nutrients and, consequently, reflects on the final quality of the fruit (Norrie et al., 2002; Khan et al., 2009; Frioni et al., 2018; Pereira et al., 2020).

Thus, increases in macro and micronutrient concentrations in corn, wheat, tomato, spinach and olive trees were reported after application of *A. nodosum* extract (Chouliaras et al., 2009; Fan et al., 2013; Sen et al., 2014; Di Stasio et al., 2018; Shukla & Prithiviraj, 2021).

The *A. nodosum* extract induces the production of glutathione synthetase, an enzyme that is responsible for converting ammonium into glutamine in an important pathway of nitrogen (N) assimilation metabolism in plants (Oliveira et al., 2002). Still in the N metabolism, the application of *A. nodosum* induces increases in the expression of nitrate reductase, another

important enzyme in the N assimilation process as it is responsible for converting nitrate to nitrite (Fan et al., 2013). Showing the importance of this compound in the absorption pathways and increase in the efficiency of nitrogen use by plants.

Nutrient concentration was evaluated in corn plants treated with *A. nodosum* extract and it was found that the levels of elements such as calcium (Ca), magnesium (Mg), sulfur (S) and molybdenum (Mo) increased by up to three times after the extract was applied when compared to the control treatment (Ertani et al., 2018). Also, according to the authors, elements such as boron (B), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) increased their concentrations, but at a lower intensity than those mentioned above.

Biological aging is nothing more than an oxidative process that causes membrane degradation associated with cell tissue death (Hodges et al., 2004). When associated with post-harvest processes, biological aging is linked to decreased enzymatic activity, loss of pigments and membrane ruptures, always resulting in quality losses (Hodges et al., 2004; Fan et al., 2014).

Literature has shown that the application of *A. nodosum* increases not only the quality of the harvested product but also the shelf life (Fan et al., 2014; Frioni et al., 2018). This work shows that the application of algae extract induces changes in physiology, mainly related to the secondary metabolism of plants, which improve shelf life (Fan et al., 2014). Among the related compounds, we can mention phenolic and anthocyanins compounds and enzymes of the antioxidant system - (e.g. peroxidases) - which slow down the degradation process and maintain the integrity and turgor of cells (Fan et al., 2014).; Di Stasio et al., 2018; Frioni et al., 2018; Melo et al., 2018).

c) Stress mitigation

Plants under stress increase the amount of reactive oxygen species (EROS) that disrupt cell homeostasis leading to apparent oxidative damage and even cell death (Zhang et al., 2016; Rosa et al., 2021). As stated earlier, plants are able to adapt to alleviate stressful situations through their adaptive responses to the environment and these responses involve physiological, biochemical and morphological changes (Taiz et al., 2017; Rosa et al., 2021).

In Brazil, the main stresses that plants are subject to are water deficit and, as a consequence, excess light; high temperatures and, occasionally, the salinity that may occur, largely due to the nutrient sources used in fertigation or in drier areas and pest and disease attacks. Bearing in mind that all these, in one way or another, resulted in the production of EROS in different parts of the plants and under different intensities.

Water deficit has a direct influence on the photosynthesis process by affecting the opening and closing of stomata, CO_2 assimilation and, consequently, low protein assimilation and sugar conversion (Santaniello et al., 2017). The result of these alterations results in high levels of EROS, mainly hydrogen peroxide and superoxide radicals.
(Santaniello et al., 2017).

To attenuate the damage caused by EROS due to lack of water are the metabolic control of transpiration, photosynthesis, increased enzyme activity (SOD, CAT, APX) and osmotic and stomatal controls (Santaniello et al., 2017; Pereira et al., 2020; Rosa et al., 2021). Remembering that most of these processes are regulated by abscisic acid. The morphological changes of roots, both in volume and in length, are also adaptation mechanisms that occur for plants to explore a greater volume of soil and have more access to water and nutrients.

Always related to the water deficit, there is excess light and the increase in respiration and transpiration to maintain the temperature of the leaves. Thus, while the absorbed light is used by the photosynthetic apparatus, thermal energy is dissipated so that it does not cause damage to chlorophylls (Santaniello et al., 2017; Taiz et al., 2017). On the other hand, when there is a surplus of light, which happens under water deficit, if this dissipation does not occur, there is an accumulation of EROS and the enzymatic mechanisms must act to mitigate the damage (Santaniello et al., 2017; Pereira et al., 2020).

During photosynthesis and respiration, there is a sequence of reactions that always involve strong electron reducers, and among the products of the reactions are superoxide radicals, hydrogen peroxides and hydroxyl radicals that are highly reactive EROS (Sewelam et al., 2016; Taiz et al., 2016; Taiz et al. al., 2017). For cells to detoxify, antioxidant metabolism must be activated and several enzymes and nutrients are necessary for this process to take place, as can be seen in Figure 2 (Sewelam et al., 2016; Taiz et al., 2017).

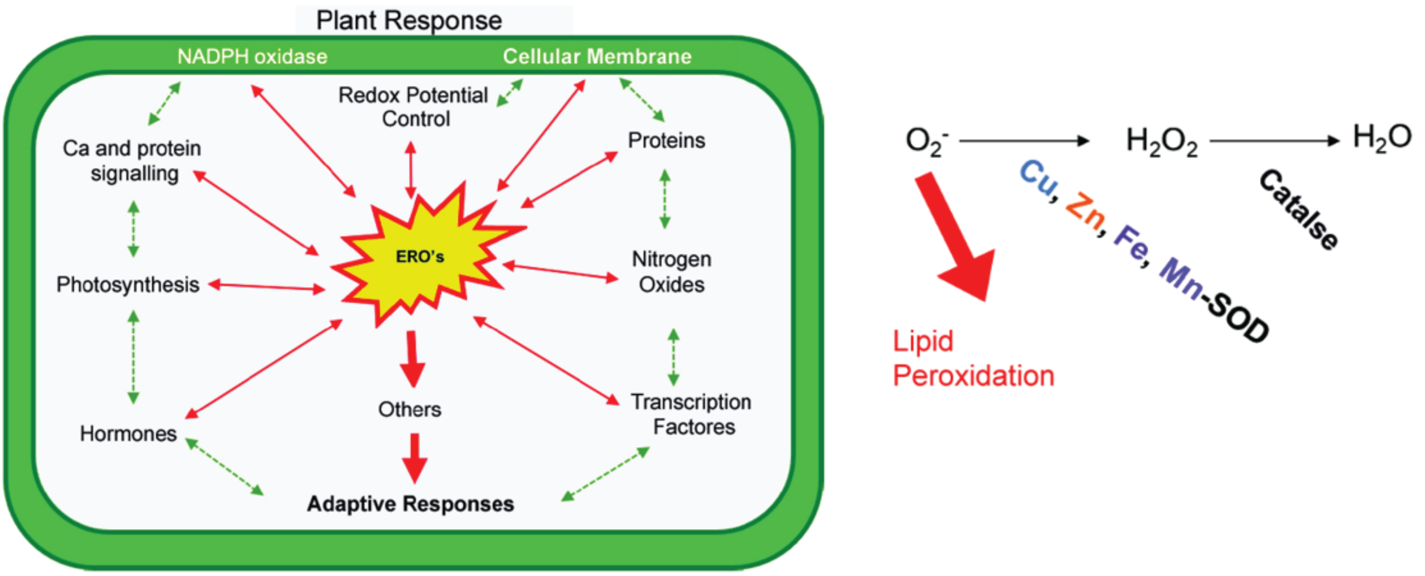


Figure 2. Sequence of events leading to the formation of Reactive Oxygen Species (ROS) in plants and enzymes involved in the detoxification process in cells. Adapted from Sewelam et al. (2016) and Taiz et al. (2017).

Applications of *A. nodosum* targeting plant tolerance to both biotic and abiotic stresses have been widely used in agriculture (Jayaraman et al., 2011; Rosa et al., 2021; Shukla & Prithiviraj, 2021).

Responses to stress attenuation have been attributed to the different methods of action that are possible through the application of *A. nodosum* (Shukla et al. 2019). The main modes of action presented are increased hormonal modulation; osmotic control; improvement in nutrient efficiency and utilization; in nitrogen and sulfur metabolism; on the activities of the antioxidant system and on secondary metabolism; conformational changes; enlargement of the root system; among others (Khan et al., 2009; Santaniello et al., 2017; Shukla et al., 2019; Pereira et al., 2020).

IMPROVER MAX

Maximum Potential in seed treatment

As seen so far, algae extracts are applied under different forms and intentions, but always with the same aim of improving the response of plants through stimuli, whether in terms of efficiency of use and improvement of the harvested product or in the attenuation of stress. Thus, **Improver Max Technology** offers the best response in the field in seed treatment for grasses, especially for corn, wheat and rice crops.

Improver Max improves seed coating and is compatible with microorganisms used in TS (Figure 3).

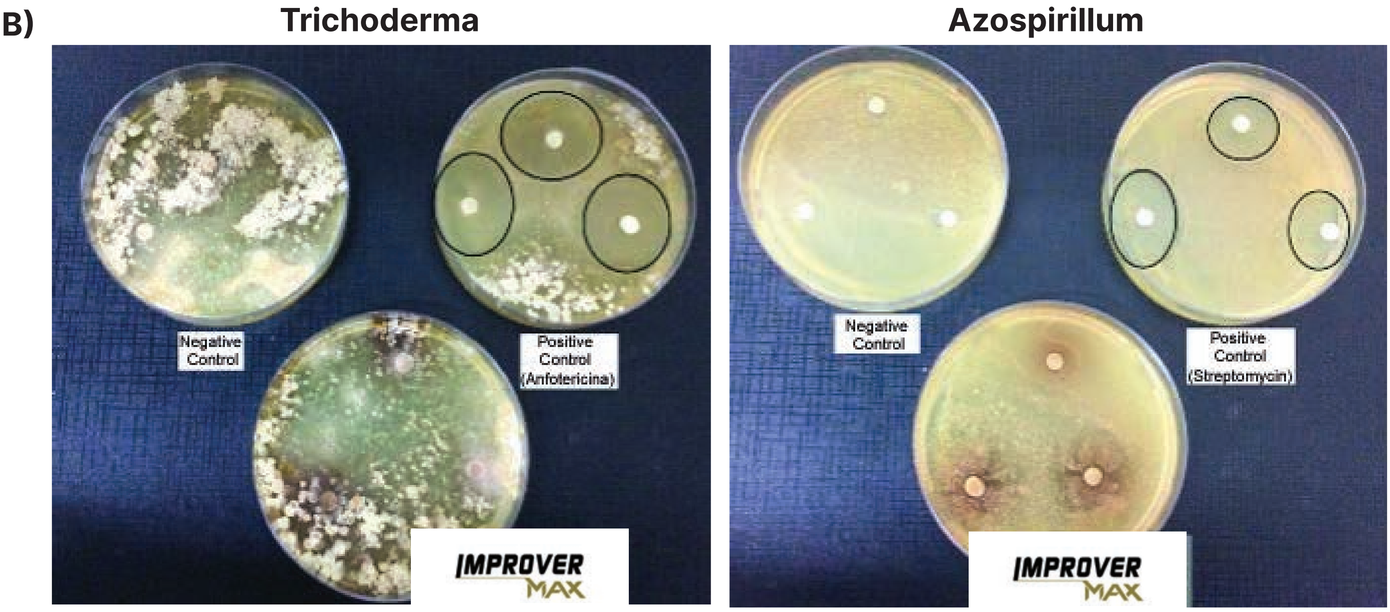


Figure 3. (A) Recoating wheat seeds, where some seeds were left uncoated in the control treatment while in the Improver Max treatment, the seeds were more uniformly treated. (B) Compatibility of **Improver Max** with Tricoderma spp. and Azospirillum spp.

In addition to better seed coating and compatibility with TS microorganisms, **Improver Max** offers a balance between physiological and nutritional effects all in one product:

I. It enhances the expression of seed vigor, promoting greater uniformity in the initial development of plants.

II. It promotes greater development of the root system, allowing a greater volume of exploited soil.

III. The formation of a deep and branched root system promoting greater physiological balance and increasing tolerance to water deficit, especially when it comes to crops planted in a rainfed system (Figure 4).

Figure 4. Control treatment plants (left) and Improver Max plants (right) at V-6 stage under dry conditions for 12 days without rain (Photo: **Innovation Center - ICL South America**).

As a result of these benefits, there is a greater accumulation of biomass in corn plants starting in the initial stages. In addition to this increase, there is also an increase in the nutrient content of the shoots of the plants in the field (Figure 5). Showing that in addition to improvements in plant metabolism, **Improver Max** also promoted greater plant growth and access to more nutrients upon emergence.

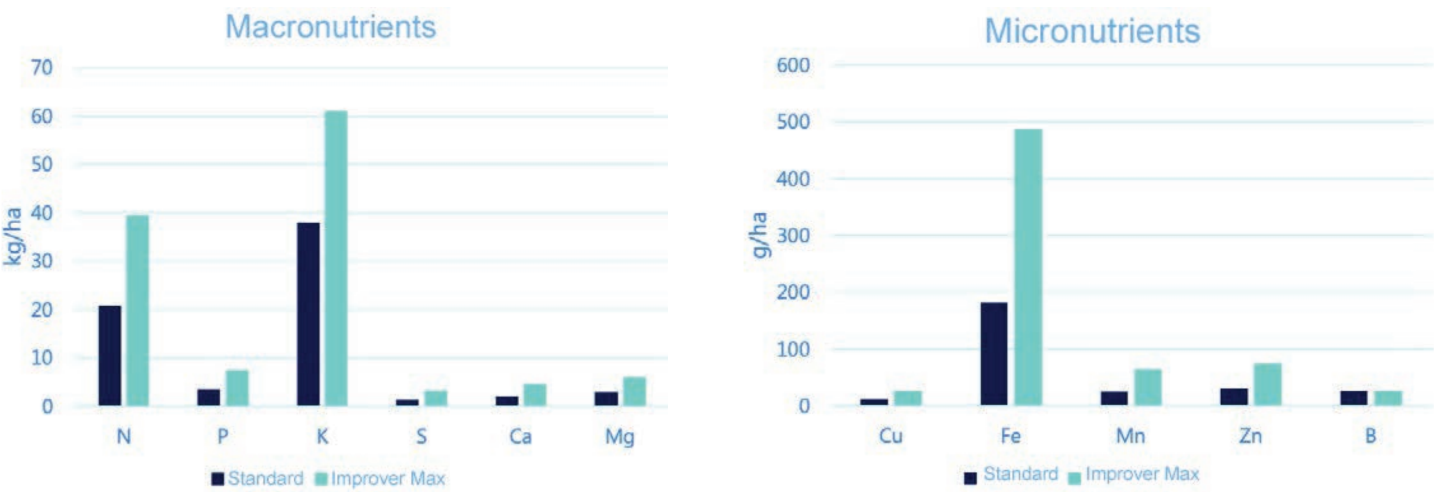


Figure 5. Nutrient accumulation in corn plants at the V6 stage. Source: **ICL´s Innovation Center South America**.

Molybdenum (Mo) plays an important role in drought resistance, as it participates in the regulation of abscisic acid, which will induce the accumulation of compatible solutes, act to close the stomata and in root growth (Marschner et al., 2012; Taiz et al., 2012; Taiz et al. al., 2017).

Improver Max increases stress tolerance in the initial moments of the crop, promoting the osmotic adjustment of the water potential of the plants without reducing turgidity and preventing dehydration (Figure 8). It is worth noting that as the soil dries, its water potential becomes increasingly negative and plants can only absorb water when their water potential is more negative than that of the soil (Taiz et al., 2017). Thus, the osmotic adjustment and, consequently, the accumulation of compatible solutes through the cells is a process that aims to protect the membranes against desiccation, replacing water molecules or preventing their exit. Leaves that can make the osmotic adjustment maintain turgidity at lower water potentials, which allow them to continue to grow and make it easier for the stoma to open (Taiz et al., 2017).

It has been observed that, in general, increase in cooling tolerance is related to an increase in the concentration of compatible solutes in the cells. The accumulated solutes are: proline, sugar alcohols (sorbitol and mannitol) and betaine.

- Osmotic effect: through vacuole accumulation, sugars decrease the amount of ice formed and can therefore increase the cell's tolerance to increased dehydration

- Water crystallization is directly related to the concentration of solutes and it is expected that the higher the concentration of solutes in the cell and the higher the presence of hydrophilic substances, the lower the lethal temperature and evidently the higher the tolerance of the cell, and considering that the amount of frozen water is equivalent to cellular dehydration, the higher the concentration of cellular juice, the lower the dehydration and consequent damage.

- Mo in ABA regulation: Mo deficient plants are more sensitive to low temperature stress, probably due to the effect of Mo on ABA biosynthesis.

Learn more about this new technology with proven results in the field (Figures 6 to 8)!

Rice crop results

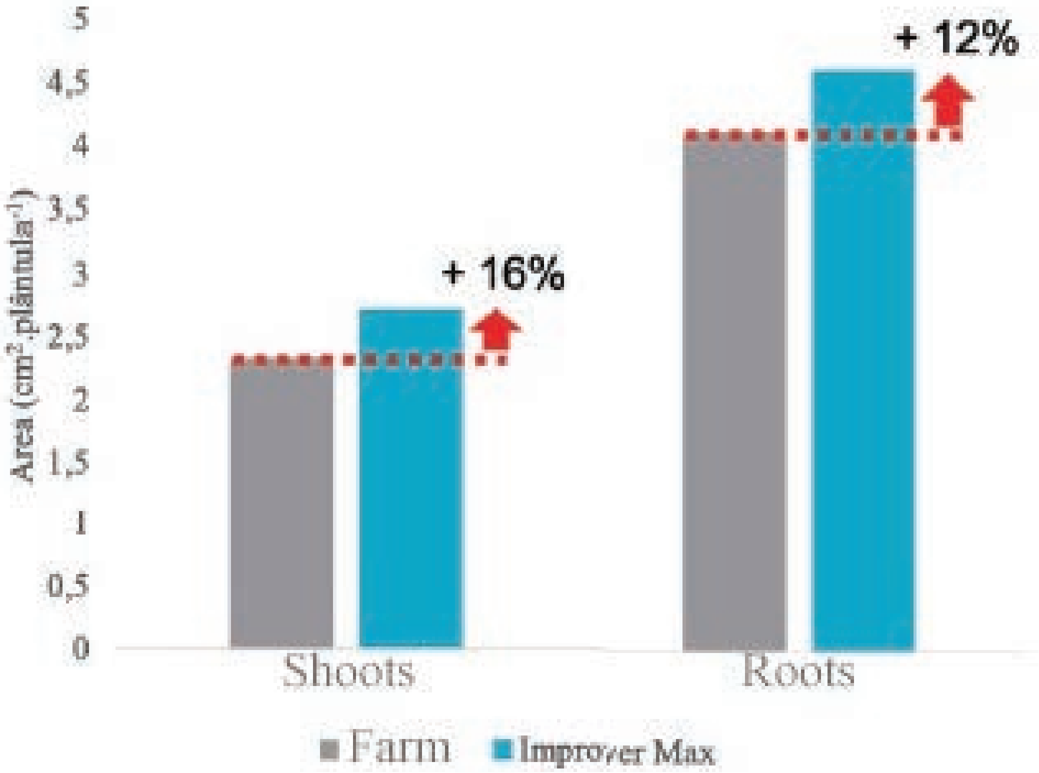
A)

B)

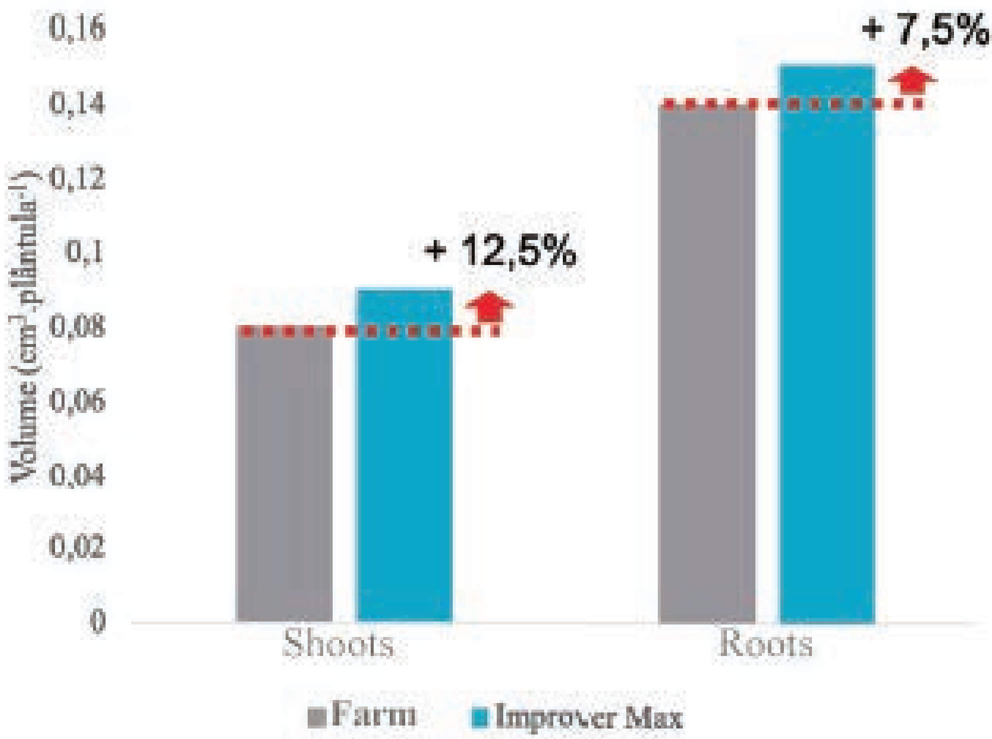
C)


18
16
14
12
10
8
6
4
2
0
Length (cm.seedling-¹)
+ 18%
+ 16%
Shoots
Roots
Farm
Improver Max

D)


5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
Area (cm².plântula⁻¹)
+ 12%
+ 16%
Shoots
Roots
Farm
Improver Max

E)



F)

G)

Figure 6. Comparison between rice seedlings under control treatment (A) and seedlings treated with **Improver Max** (B); shoot and root length (C), shoot and root area (D) and shoot and root volume (E) of rice seedlings at the V3 stage. Productivity results in irrigated rice fields (F and G).

Corn crop results

A)

B)

C)

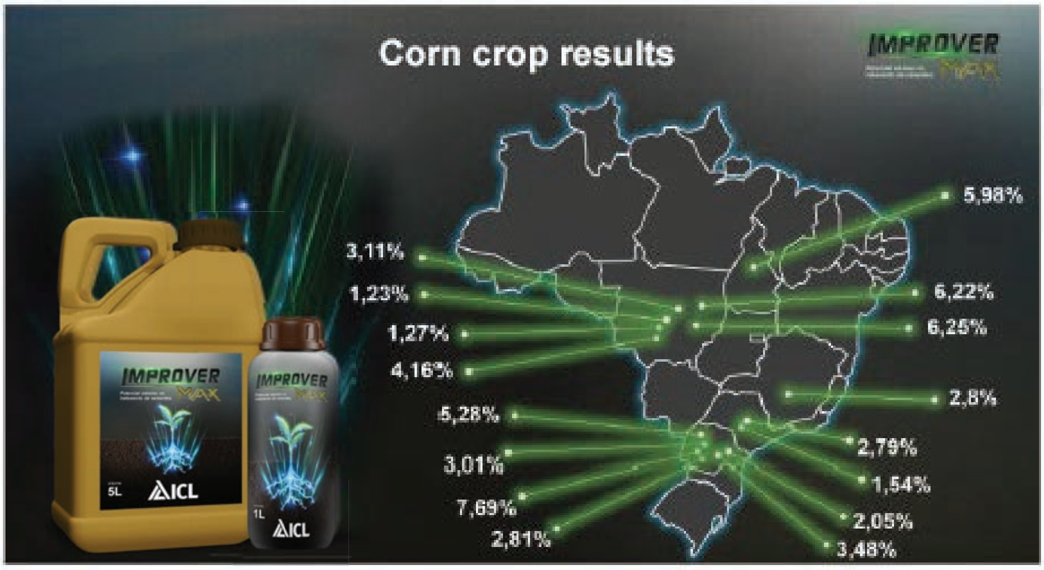


Figure 7. Comparison between corn seedlings under control treatment (A) and seedlings treated with **Improver Max** (B); Productivity results in cornfields conducted across the country (C).

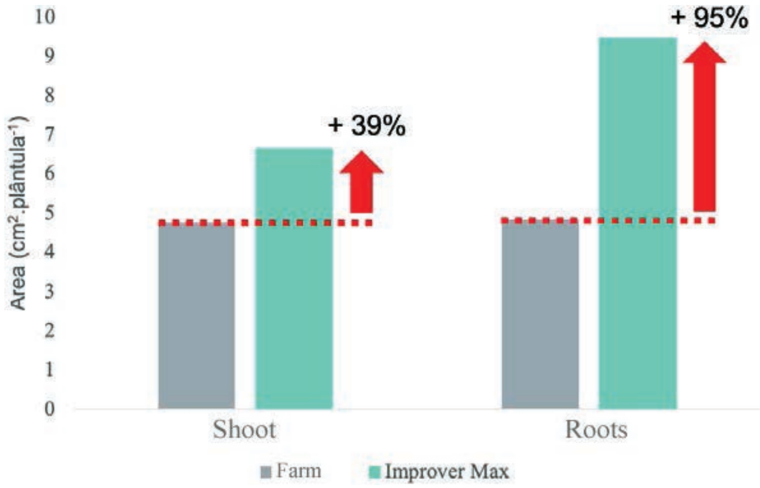
Wheat crop results

A)

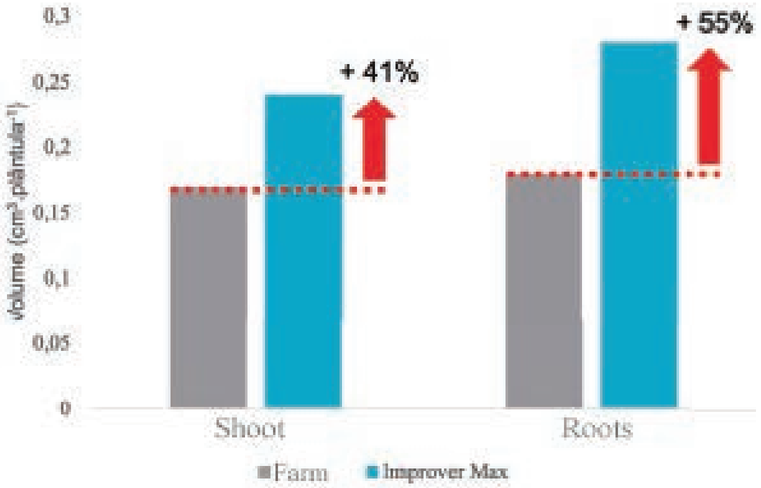
B)

C)

D)



E)



F)

Figure 8. Comparison between wheat seedlings under control treatment (A) and seedlings treated with **Improver Max** (B); shoot and root length (C), shoot and root area (D) and shoot and root volume (E) of wheat seedlings at the V3 stage. Productivity results in wheat fields (F).

IMPROVER MAX

Maximum Potential in seed treatment

Recommended Dose:

Rice: 1.0 mL/kg of seed
Corn: 100 mL/ha
Wheat: 1.5 mL/kg of seed

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