Full Length Research Paper

**Kappaphycus alvarezii** extract used for the seed treatment of soybean culture

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Marine macroalgae, **Kappaphycus alvarezii** has economic importance which is considered as the main source of **Kappa carrageenan**, a hydrocolloid used in various industries as an emulsifying agent and stabilizer. In the production process of this raw material, the fresh seaweed is subjected to grinding and filtering process, yielding a wet solid fraction rich in carrageenan and other liquid fractions. The latter has been studied for its use in pulverization of bean leaves, wheat, soybean, rice, among others. Several reports have shown positive effects on the growth, yield and quality of vegetables and grains treated with the extract of this alga. However, there are few reports on the use of the liquid extract of **K. alvarezii** for seed treatment, and also of its use in conjunction with the solid fraction of seaweed. In this context, this study aims to evaluate the physiological quality of soybean seeds treated with liquid extract of this alga and, with the mixture of liquid extract and solid biomass, submitted to a hydrolysis process. Two soybean seed treatment tests were performed in laboratory: one treatment contained pure liquid extract (100%) while the other was mixed with two samples of solid biomass hydrolyzed with concentrations of 25 and 75%. For comparison of their results, there was a positive control treatment and one negative control treatment. The results showed that, within the present working conditions, treatments with seaweed extract can increase the length of the aerial part of the seedling up to 28% and the length of the primary root up to 19% as compared to the negative control; thus, it improves the physiological performance of seeds.

**Key words:** Glycine max, macroalgae, agriculture, bioregulator.

INTRODUCTION

**Kappaphycus alvarezii** (Doty) Doty ex P.C. Silva is one of the main algae grown in the world. Along with other red algae, **Eucheuma** are responsible for about 34% of world production of seaweed (FAO, 2012). The commercial...
importance of these organisms is because it is the source of carrageenan, a phycocolloid which has many industrial applications due to its emulsifying and stabilizing properties (Yong et al., 2013). The cultivation technique mastery and the increase in world demand of this phycocolloid made *K. alvarezii* widely cultivated in several countries (Ask and Azanza, 2002).

The introduction of this species in Brazil took place in 1995 in experimental scale cultivation in Northern São Paulo State coast and aimed to make the country self-sufficient in the production of carrageenan. From this moment, several studies have been conducted to evaluate the commercial viability of marine farms of *K. alvarezii*, but despite the acknowledged progress in this matter, Brazil is not yet part of the world's leading producers and imports most of its domestic demand of carrageenan and agar, primarily from Philippines and Chile (Paula et al., 1998; Oliveira et al., 2009). However, today, *K. alvarezii* is the only marine macroalgae legally cultivated in Brazilian coast.

In addition to the global demand for carrageenan, potential new biotechnologies are being used to find these species of seaweed and its derivatives to obtain new products, and increase phycocolloid. This will encourage the cultivation of the macroalgae. An example is the work carried out to evaluate its antimicrobial potential (Abirami and Kowsalya, 2011; Prabha et al., 2013; Pushparaj et al., 2014), the profile of its nutritional and biochemical composition (Fayaz et al., 2005; Chew et al., 2008; Abirami and Kowsalya, 2011) and the use of its extract in agriculture (Rathore et al., 2009; Zodape et al., 2009, 2010).

In particular, the use of seaweed extract in agriculture has shown interesting results in the increment of growth, yield and quality of some crops such as soybeans, wheat and beans (Rathore et al., 2009; Zodape et al., 2009, 2010). In general, the use of algae in agriculture has occurred for centuries; however, they have currently been used specifically as biostimulants or bioregulators, for foliar application and/or seed treatment. These compounds are able to act on the physiology of plants, where a number of biomolecules perform metabolic functions that contribute to a better development of the plant. Among the biomolecules usually present in these extracts, the plant hormones, such as abscisic acid, auxin, cytokinins and gibberellins can be highlighted among others (Craigie, 2011).

In literature, there are results that demonstrate the effectiveness of products with growth promoter or promoter action, as is the case of stimulant. The results obtained by the authors (Vieira and Castro, 2001; Ávila et al., 2008) with the product, or other regulators are applied exogenously. Such research has attested that there is a real possibility of bioregulators to not only change the agronomic performance of soybeans, but also to produce seeds with different physiological potential and incidence of pathogens, as well as seed treatment, to promote better initial growth, which contributes to a better development of culture and derivations, best yields (Marcos Filho, 2005).

In addition to the use of liquid seaweed extract, studies suggest other applications for solid biomass of *K. alvarezii* like acid hydrolysis process that can serve as raw material for bioethanol production. Although, the primary application for the acid hydrolysis of biomass is the production of raw material to obtain bioethanol, there are several biological activities related to oligosaccharides derived from molecules such as carrageenan, which may appear as other applications, such as antiviral (Katsuraya et al., 1999), antitumor (Yuan et al., 2006), anti-inflammatory (Sanders et al., 1999), anticoagulants (Wall et al., 2001) and antiangiogenic (Käsbaier et al., 2001).

In this context, considering the proven potential of the liquid extract of *K. alvarezii* in agriculture and the possible biological activity of the polymers present in the solid fraction of the algae and its derivatives, the common use of these biomasses can expand the positive physiological responses already obtained in vegetable treatment with the seaweed liquid extract. And considering that these liquid extracts contain products with potential hormonal action, the present study aimed to evaluate the use of liquid extract of *K. alvarezii* and also its use in conjunction with the liquid extract and algae hydrolyzate solid biomass, in soybean, for verification of their effects in the physiological quality of the seeds.

### MATERIALS AND METHODS

Two seed treatment trials were conducted at the Seed Technology Laboratory, Department of Agricultural Sciences, Federal University of Parana, Palotina Sector.

The extracts used in the work are derived from seaweed cultivated in Brazil coastline with the tubular net technique. They were obtained according to the method described by Eswaran et al. (2005). After harvesting, the algae were subjected to the following processes: washing in running water in order to remove any residue or marine impurity; mechanical trituration with industrial shredder to break the cell wall and release the sap; filtration of the aqueous biomass through a cotton cloth, then obtaining two biomass, liquid extract and a moist solid phase. The wet solid phase was exposed to sunlight for a period of approximately 72 h, until the moisture content reached a percentage (%) below 25 and it was stored in a suitable place for its conservation. The liquid phase was bottled and stored in the proper location for conservation.

After grinding and sieving, the solid biomass of algae was subjected to acid hydrolysis. The process was carried out using an autoclave and glass jars with capacity of 500 mL, where the working volume was approximately 200 ml employing a seaweed substrate concentration of 12 g 100 ml⁻¹ solution of sulfuric acid. The concentration of the sulfuric acid solution was 0.2 molar, with reaction temperature of 127°C and two times reactions of 15 and 30 min. This resulted in samples called solid biomass hydrolyzate 1 and 2, respectively.

For each seed treatment test, the experimental design was completely randomized with twelve treatments, of four replications. In each case these was analyzed: one negative control treatment (T0): a control positive treatment (T1) consisting of the commercial...
product Stimulate®, a plant growth regulator promoters-based: cytokinin, auxin and gibberellin (kinetin, as gibberellic acid GA3 form; 4-indol-3-ibutirico, respectively); two treatments with pure liquid extract (T2 and T3); two treatments with the solid biomass hydrolyzed 1 (one) in mixture with the liquid extract in concentration of 25% (T4, T5); two treatments with the solid biomass hydrolyzate 1 in mixture with the liquid extract in concentration of 75% (T6, T7); two treatments with the solid biomass hydrolyzate 2 in mixture with the liquid extract in concentration of 25% (T8 and T9); and two treatments with the solid biomass hydrolyzate 2 in mixture with the liquid extract in concentration of 75% (T10 and T11).

The two trials only differ in the groups of doses used, which in the first test treatment with seaweed had two doses of 2.5 and 5.0 mL for 100 kg of seed. The negative control consisted of no treatment and positive witness the Bioregulator Stimulate® with dose of 5.0 mL for 100 kg of seeds. And, for the second experiment, the doses of treatment with seaweed were 4.0 and 8.0 mL for 100 kg of seed, also constituting negative control of no treatment, and the positive had the Bioregulator Stimulate®, with dose of 8.0 mL for 100 kg of seed. The soybean cultivar used for the seed treatment was Monsoy 5947 IPRO.

To evaluate the physiological quality of the seeds, the percentage of germination was determined, performed with four subsamples of 50 seeds each, with the volume of water equivalent to two and a half times the mass of the dry paper. The rolls were placed in germinator Mangelsdorf, at constant temperature of 25 ± 2°C. The percentage of normal seedlings, according to the Rules for Seed Analysis (MAPA, 2009), was analyzed on the eighth day at the beginning of the experiment. The performance of seedlings was also evaluated through the length of the seedlings, using four replications of 20 seeds for each treatment, germinated in the same conditions of the germination test. The seeds were distributed in the longitudinal direction of the sheets, with the thread facing the lower end of the substrate. The rolls were placed vertically in the germinator, at a temperature of 25 ± 2°C. The length of normal seedlings (primary root and aerial part) was assessed on the seventh day, with the aid of a millimeter ruler, getting the results in cm/seedling (Nakagawa, 1999).

Finally, the biomass of the average dry weight (g of seedling) of the seedlings after the length evaluation was determined. Normal seedlings were placed in paper bags and dried in an oven with forced-air circulation, at 80 ± 2°C for 24 h and then the weigh in analytical balance was held (Nakagawa, 1999).

The effects of the treatments were checked by analysis of variance and the averages compared by Tukey test at 5% significance through the Sisvar® computer program.

RESULTS AND DISCUSSION

According to statistical analysis, the results obtained in the first assay, doses varied between 2.5 and 5.0 mL for 100 kg of seed, and are shown in Table 1. Some treatments showed significant differences for aerial part length parameters and primary root. According to the results, only the treatments derived from the mixture of liquid extract with solid biomass hydrolyzate presented statistics superior to the negative control (T0) for the length of the aerial part of the seedlings. However, these treatments did not differ significantly among themselves and with the positive control (T1). On average, treatment with lower dose and lower concentrations of liquid extract (T10), presented an increase of more than 28% as compared to the negative control (T0).

Regarding the length character of primary root, only two treatments showed significant difference as compared to the negative control (T0), which is the treatments with lower dose and lower concentrations of liquid extract (T6 and T10), with higher increase to 11%. However, they did not present significant difference between themselves and in relation to the positive control (T1).

The results of the second assay, where the doses vary ranging between 4.0 and 8.0 mL for 100 kg-1 of seed, are shown in Table 2. Similar to the first experiment, some treatments showed significant difference only for the shoot variable length and primary root. In this case, considering the results of the aerial part length, only the treatments derived from the mixture of liquid extract with the hydrolyzed biomass were statistically higher than the negative control (T0), an increase of up to 23%. However, they did not present significant difference between themselves and in relation to the positive control (T1).

Finally, in the case of aerial part length, only the treatment with lower dose of pure liquid extract (T2) and the positive control (T1) showed a significant difference as compared to the negative control (T0), with an increase of up to 19%. However, they did not present differences when they were compared.

Considering the possible content of phytohormones (or similar) of the liquid extract, these results are consistent with the report of Moterle et al. (2011) and Vieira and Castro (2001) who found that there were positive changes in the performance of seedlings, increase in the length of the aerial part of the soybean seedlings treated with Stimulate®. One was Bioregulator registered and recommended for soybean culture (EMBRAPA, 2014). And, also with the results obtained by Santos (2009), where the same plant growth regulator was able to promote the initial growth of soybean plants, increasing the average height. Further, Wang et al. (2008) observed another effect, that there was a delay in the emergence and early development of soybean seedlings when treated with high doses of gibberellin and cytokinin. However, the results tend to show promoting answers. For instance, França-Neto et al. (2011), using biostimulants seaweed extract derived from the soybean seed treatment with micronutrients, reported positive effects on seedling length.

The literature has several reports of significant expressive effects of commercial bioregulators (Klahold et al., 2006; Ávila et al., 2008), as well as the potential use of new substances, associated or not with nutrients and phytosanitary products (Ferreira et al., 2007; Castro et al., 2008). This emphasizes the need for continuing the researches, seeking alternatives not only for the best physiological performance of the seeds, but also for the culture as a whole, in terms of agronomic yields.

In this context, the results suggest the biostimulant potential of the K. alvarezii extract, which showed...
tendancy to increase length of soybean seedlings, where the use of some products of the extract have evident technological potential.

Conclusion

The use of seaweed, \textit{K. alvarezi} extract can improve the
physiological quality of soybean seeds, providing the best performance seedlings. Some products were superior and similar to commercial bioirrigulator. However, more studies are necessary to understand the real promoter effect of extracts and their preparations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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