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Microalgae as growth promotors (biostimulants) in *Triticum aestivum* L.

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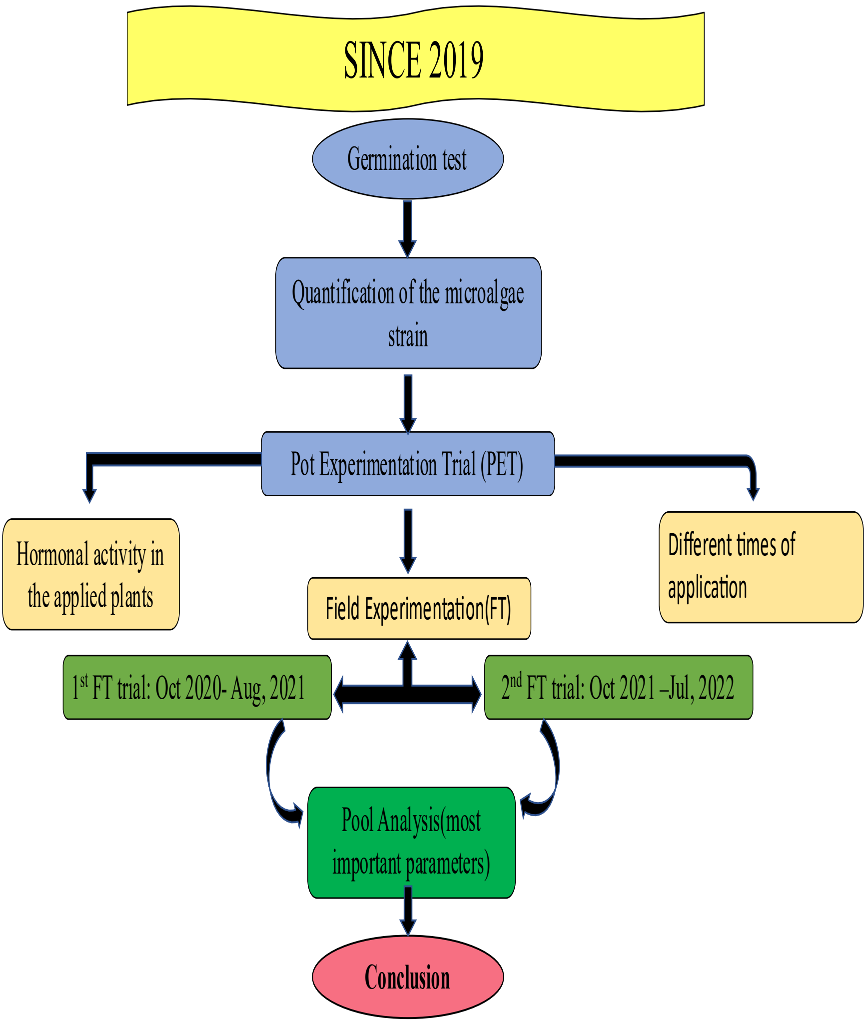
SINCE 2019
Germination test
Quantification of the microalgae strain
Pot Experimentation Trial (PET)
Hormonal activity in the applied plants
Field Experimentation(FT)
Different times of application
1st FT trial: Oct 2020- Aug, 2021
2nd FT trial: Oct 2021 –Jul, 2022
Pool Analysis(most important parameters)
Conclusion

Introduction

As an agriculturist, many researchers have been trying to establish potential uses of microalgae, how and in what ways. In plants, application as a biofertilizer, biostimulant, or biocontrol agent, may change the biochemical processes. More recently, the new Regulation (EU) 2019/1009 (Rouphael and Colla, 2020) has defined PBs as follows: “A plant biostimulant shall be an EU fertilizing product the function of which is to stimulate plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: i) nutrient use efficiency, ii) tolerance to abiotic stress, iii) quality traits, or iv) availability of confined nutrients in the soil or rhizosphere” (EU, 2019). Based on the statement, our aim and objective were framed to confirm the potentiality of microalgae as biostimulant or plant regulator.

Aim and objectives: The main aim of the present work was to determine the possible mode of application of certain algal strains in wheat plants at different developmental stages and different growth conditions. Particularly, the following objectives were in focus:

1. To quantify the secondary metabolites, present in the selected microalgae.
2. To study the hormonal activities in the microalgae-applied wheat leaves.
3. To study the physiological and morphological parameters of the crop following the application of different microalgae at germination and seedling stages under greenhouse and field conditions.
4. To study the biochemical properties of the microalgae as well as the effect on biochemical properties of wheat.
5. To examine the effect of microalgae treatments on wheat quality.

Materials and methods:

The research was conducted at different forms, including preliminary laboratory trials, pot experiment, and field experiments. Initially for the preliminary trial, the biomass of strains MACC-922 (*Chlorella vulgaris*), MACC-612 (*Nostoc linckia*), MACC-683 (*Nostoc* sp.), MACC-755 (*Chlorella vulgaris*), MACC-430 (*Chlamydopodium fusiforme*), MACC- 677 (*Tetradesmus obliquus*), MACC-519 (*Chlorella sp*) and MACC- 438 (*Chlorella sorokiniana*) after producing in laboratory conditions fulfilling all standard requirements were used for germination test. In the mungbean bioassay the strains tested were reduced to MACC-612, MACC-922, MACC-430, and MACC-438 (Hess (1961 method was used)). The same four selected strains were quantified using an Acquity I/Class UPLC system - Xevo TQ/XS tandem mass spectrometer quantification. The method was explained by Hrdlička et al (2019).

A total of 6 pot experiments were conducted in vernalized and non-vernalized seedlings (Mv Nádor and Mv Béres varieties) Three independent pot experiments were conducted. In Trials 1 and 2 Mv Nádor, in Trial 3 Mv Béres variety was used. Three strains of algae were tested in each trial, MACC-612 (*Nostoc linckia*), MACC-430 (*Chlamydopodium fusiforme*), and MACC-922 (*Chlorella vulgaris*).

In Trial 1 the application timing was at the critical flowering stage or early reproductive stage, in Trials 2 and 3 algae treatments were applied at the early vegetative stage.

In another pot experiment, non-vernalized wheat plants were sprayed. These were mainly to check hormonal activity. 2 pot experiments were used for determining photosynthetic activities.

Field Experimentation

Trt	Code	Description
Trt 1	CHK	Untreated check
Trt 2	Standard CHK	6-BAP@
Trt 3		MACC-612
Trt 4		MACC-612 + Trend 90
Trt 5		MACC-430
Trt 6		MACC-430+ Trend 90

Trt 7		MACC-922
Trt 8		MACC-922 + Trend 90

Randomized block design with 4 replications was used(variety: Mv. Kunhalom) Foliar spray was conducted at Critical flowering stage spraying at 1g/ha conc. Spray volume: 300 L/ha.

Parameters/Observations

Parameters like Chlorophyll and carotenoid content (Arnon, 1949), Hexose sugar estimation **(**DuBois et al. 1956), estimation of total polyphenol content **(**Singleton and Rossi 1965), Ferric reducing antioxidants power (FRAP) assay (Benzie and Strain 1999), Proline content estimation (Bates 1973) were assessed.

Fertility % = No. of fertilized spikelets (seeds)/ total number of floral primordia × 100 %

Sterility %= No. of unfertilized spikelet/total number of floral primordia × 100 %

Number of unfertilized spikelets = Total number of floral primordia-No. of fertilized spikelets

Other parameters like Crop Growth Rate (g/m^2/day, Relative Leaf Water Content (%), Number of tillers,1000 kernel weight, no. of grains per ear, above-ground biological yield or straw yield, grain yield in kg/ha, Harvest Index (HI), total nitrogen (%), nitrate and nitrite content, nitrogen use efficiency, were some parameters taken.

Post-harvest, grain analysis was used to determine the protein, gluten, and Zeleny sedimentation value and the ratio of gluten proteins, the glutenins, and the gliadins (Glu/Gli), the amount of unextractable polymeric proteins (UPP) were found out.

Results and discussion

The biomass of algae strains like MACC-612 (*Nostoc linckia*), MACC-430 (*Chlamydopodium fusiforme*), MACC 922 (*Chlorella vulgaris*) showed significant differences with the control compared to other strains, so these three strains can be upgraded for the field experiment.

Certain algae strains improved, while others, e.g., MACC-438 (*Chlorella sorokiniana*), inhibited the germination processes. However, the way they affected germination may not work in the same way when they were used via leaves. What inhibits germination does not necessarily have an inhibitory effect on adult plants. A significant proportion of the strains was characterized by auxin-like activity. However, the auxin-like effect was not necessarily in direct relationship with the auxin content, but with their ability to influence secondary metabolism. Some of the metabolites detected could be the reason behind significant differences in the physiological parameters. The application of microalgae biomass tends to decrease IAA and enhance metabolites such as salicylic acid, jasmonic acid conjugates, and p-HBA. However, the differences are unstable and need more trials involving different genotypes for confirmation.

Overall, there were no extremely significant differences between the treatment and the control in all the parameters however we found potential in the treatment. After analysing the results of all three trials, we can conclude that microalgae biomass application affects certain physiological and biochemical properties but still works must be done to improve the absorption or uptake of the microalgae biomass in the plants. One suggestion could be earlier spraying or increasing the number of sprays as we show potential differences in plants treated at an earlier stage, i.e., the vegetative stage.

Finally, the metabolomic analysis conducted independent of the time of application, suggested the influence of the microalgae strains in the biochemical composition of the plants.

Overall, no outstanding response was observed on many parameters, even so, we cannot ignore the fact that there was some effect of the treatment or the microalgae biomass. Our main aim was to conclude if any of the microalgae strains can be used as biostimulants or growth promotors as per Regulation (EU) 2019/1009 on Fertilising Products (FPR). After considering some of the many parameters we can verify in the following way:

1) Proline concentration, which is a sign of abiotic stress tolerance potentiality, showed some increase in

concentration after treatment of the biomass (esp. MACC-922) upon comparing with control.

2) Nitrogen use efficiency (NUE) or nitrogen uptake showed negligible difference with control. MACC-922 performed better than the other strains.

3) Quality traits such as the antioxidant potential (FRAP and TPC), protein %, gluten %, Zeleny sedimentation value, and the gluten subunits show some effect of the treatment esp. of MACC-612.

Based on the three points we can say that the microalgae strain, MACC-922, *Chlorella vulgaris* has the potential to promote or stimulate growth better than the other two, MACC-612, *Nostoc linckia* and MACC-430, *Chlamydopodium fusiforme*. However, to enhance their effectiveness it needs further improvement in the product by adding adjuvants as we found differences between treatments with or without Trend 90 (adjuvant).

NEW (NOVEL) SCIENTIFIC RESULTS OF DOCTORAL RESEARCH

- We determined the effect of the eight algae strains, i.e., MACC-922, MACC-612, MACC-683, MACC-755, MACC-430, MACC-677, MACC-519, and MACC-438 on germination and identified those that stimulate and inhibit germination processes in wheat plants. We found that the effect on germination is not necessarily the same as the effect on the development of the mature plant.
- We quantified secondary metabolites in the selected strains of microalgae biomass which revealed the presence of secondary metabolites such as salicylic acid, p-hydroxybenzoic acid, benzoic acid, etc.
- We have shown that the effect of algal treatments by foliar application on the metabolite composition of winter wheat can be influenced by factors, like, the variety and the algal strain.
- We found variability in few metrics, such as biological yield, nitrogen content in the grain, because of the differences in the time of application i.e., early vegetative and early reproductive stage.

- We concluded that genetic variability or variety played a negligible role in the effectiveness of microalgae biomass treatment on biological yield , hexose content and total phenol content.
- Observing the biochemical properties such as ferric reducing antioxidants power (FRAP) activity, total phenol content, and hexose content at different stages was the detailing added besides the common observations of proline content.
- Observations on nitrogen content in leaves, grain, and soil revealed the potentiality of microalgae in influencing the mobilization of nitrogen in the plant. Such potentiality has been reflected on nitrogen use efficiency, nitrogen uptake, etc.
- We observed the effect of microalgae biomass application on the content of gliadins, glutenins, glutenins:gliadins ratio and unextractable polymeric protein (UPP) %.

CREDENTIALS

Papers published:

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Lectures delivered:

"Action flicks of Microalgae in Triticum aestivum" presented on 10 January 2023 in the Dr. Har Gobind Khorana International Young Scientist Lecture Series-II.

"Is drainage as important as irrigation" delivered on 20 July 2020 in one-week online lecture series on Agricultural practices and approaches.

Conference materials as abstract

1. Mutum Lamnganbi, Kabato Wogene, Ördög Vince, Tibor Janda, Molnar, (2021). Secondary metabolites of microalgae and thier relationship with germination and hormonal activity in winter wheat, Abstract book-XIII. HUNGARIAN PLANT BIOLOGY CONGRESS Biological Research

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3. Mutum Lamnganbi, Wogene Kobato, Tibor Janda, Zoltan Molnar (2022): Chitosan and Microalgae stimulators compensating deprivation of early physiological and biochemical development of winter wheat at half N-portion. In: C. Jacquard, E. Ait-Barka, C. Clement (Eds.) Plant BioProTech 2022, 27-30 June 2022, Reims, France, Poster Abstracts, p. 31.

4. Mutum Lamnganbi, K.P.Sharma, Pinky Goyal, Mahendru Gautam, Zoltan Molnar (2020).Yield performance of transplanted quinoa in deficit irrigated condition.In abstract book:19th Alps-Adria Scientific Workshop, 26th April-1st May,2020, Wisla,Poland, Abstract: p. 68.