Bangladesh J. Bot. **51**(2): 255-262, 2022 (June) DOI: https://doi.org/10.3329/bjb.v51i2.60422

IMPACT OF *SCHAROMYCES CEREVISIAE* MEYEN EX E.C. HANSEN EXTRACT ON SOME GROWTH PARAMETERS OF *PISUM SATIVUM* L.

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Keywords: ADP, Gene expression, NTP, *Pisum sativum*, Yeast extract

Abstract

Some growth biomarkers of germinated pea (*Pisum sativum* L.) using 1% yeast (*Saccharomyces cerevisiae* Meyen ex E.C. Hansen) extract was investigated. Seeds of pea were cultivated in the clay soil and irrigated by 1% yeast extract and/or foliar spray for 20 days. The highest values of 49 kDa apyrase, NTP, ADP substrate analysis, amino acids content, protein content and photosynthetic pigments were recorded from an addition of yeast extract to the soil along with foliar spray. NTP and ADP substrate activities analysis in the leaves were found to increase between 11.69 and 0.13%, 49 kDa gene expression between 15.77 and 0.86%, amino acids and protein contents between 40.15 and 2.85% and photosynthetic pigments between7.80 and 1.54%, when yeast extract was added to the soil or/and in the form of foliar spray then effect was more than the untreated control. Yeast extract may be used as a natural bio-fertilizer instead of harmful synthetic chemicals.

Introduction

Pea (*Pisum sativum* L.) is considered as one of the main common leguminous crops grown for regional consumption as the pea pods contain high amount of protein and carbohydrates. Therefore, pea is known as important food sources for human nutrition. Fertilizers may be either bio-fertilizers or chemical and both play an important role on plant growth and productivity. For example, nitrogen is vital for chlorophyll, enzyme, and protein synthesis, phosphorus for phospho-proteins, root growth, phospholipids, ADP and ATP formation and potassium for enzyme function and improves translocation and protein synthesis (Yadav *et al.* 2005).

Although chemical fertilizer increases plant growth and vigor and fulfil the world's food safety, but plants grown in this manner does not grow in a proper way whereas root and shoot systems and nutritional characteristics have no appropriate time to fulfil plants requirement. The harmful impact of synthetic fertilizers will start from the production of these chemicals, such as NH_4, CO_2, CH_4 etc., which are causing air pollution and gradual accumulation in the human body. Fertilizers also contain a variety of heavy metals that degrade soil's physical and biological features, as well as plant development, physiological, and biochemical activities (Nagajyoti *et al.* 2010). Only by using bio-fertilizers the negative impact of these chemicals on human health and to the environment could be significantly reduced or totally eliminated. Inputs of bio-fertilizers will create a positive natural habitat for the present and for future generations to enhance plant productions (Patel *et al.* 2014).

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There is an interest in utilizing the benefits of plant-growing microorganisms for sustainable agricultural production. It was reported that microorganisms could promote plant growth and health through some mechanisms including the supply of biologically fixed nitrogen, phytohormones, volatiles and defensive compounds (Lugtenberg and Kamilova 2009). Yeast suspension was considered as a natural bio-stimulant in plants for both vegetative parts and generative stages (Ibraheim 2014). *Saccharomyces cerevisiae*, a single celled fungus has been used in wine making, baking, and brewing since ancient times. It is believed to have been originally isolated from the skin of grapes. It is one of the most intensively studied eukaryotic model organisms in molecular and cell biology, same like *Escherichia coli* as the model bacterium. The inclusion of dead or alive yeast to the soil significantly increased the nitrogen and phosphorus contents of root and shoot systems of many plant, for example, *Solanum lycopersicum* and young plants of *Saccharum officinarum* (Lonhienne *et al.* 2014). The introduction of yeast to soil also raised the ratio of root/shoot in these species that caused species-specific morphological modifications in the plant tillering and in shoot biomass.

Furthermore, it had been shown that dicotyledonous species could use yeast cells as an efficient nutrient sources, such as *Arabidopsis thaliana* and *S. lycopersicum* since they may take non-pathogenic *S. cerevisiae* and *E. coli* into root cells then digest these microbes and used them as food sources (Paungfoo-Lonhienne *et al.* 2010). It was reported that spraying pea plants with a 2% yeast extract solution improved the vegetative growth and yielded the highest yield of pods, nutritional value, enzyme activity, fresh weight, foliage dry matter and total numbers of pods/plant (Mahmoud *et al.* 2013). Some studies had also confirmed that various yeast strains improved plant growth characteristics like solubilization of phosphate (Amprayn *et al.* 2012), resistance to stress (El-Zohri *et al.* 2017) and pathogen management (Ibrahim and El-Fiki 2019).

However, to date, no study has been explored whether the bread yeast (*S. cerevisiae*) can induce the expression of 49 kDa apyrase, NTPase, ADPase in association with amino acids, protein contents and photosynthetic pigments. Therefore, the present work was undertaken to examine the impact of the addition of yeast extract to the soil and/or foliar spraying in some physiological parameters of growing pea (*P. sativum*) in clay soil.

Materials and Methods

For sowing the pea seeds (*P. sativum*), 1% yeast extract/2KG of clay soils had been prepared in pots (30 cm in diameter and 20 cm deep). Treatments were arranged as follows 1) Pea seeds were sown in the clay soils without yeast extract and irrigated by distilled water only (control); 2) Pea seeds were sown in the clay soils mixed with yeast extract and irrigated by1% of yeast extract twice a week (YE + Soil); 3) Pea seeds were sown in the clay soils mixed and irrigated by yeast extract combined with foliar spraying twice a week after 5 days of seeds germination (5 ml of 1% yeast extract for each pot) (YE + Soil + Foliar); 4) Pea seeds were sown in the clay soils without yeast extract and sprayed twice a week with 5 ml of 1% yeast extract for each pot (YE + Foliar). Treatments were arranged in triplicates and the leaves were harvested after 20 days from sowing the seeds (1stSeptember 2019, 23 to 17°C). Five plants from each pot were taken at random to evaluate amount of 49 kDa apyrase, NTPase, protein and amino acid contents and photosynthetic pigments in the leaves.

The 10 g of mature leaves with no visual damage were collected from 20-days old planted pea. Collected leaves were grounded in a cytoskeleton-stabilizing buffer (CSB) on dry ice using a mortar and pestle (Moustafa *et al.* 2003). Miracloth (Calbiochem) of two layers was used to filter the resulting homogenates that used for further research.

Leaves of 20-day old pea were harvested from each treatment, homogenized in cytoskeleton-stabilizing buffer (CSB) and then filtered through Miracloth. NTP, ADP and AMP substrates analysis activities were calculated as phosphate released from each of treatment. The 1.5 µl of leaves homogenate diluted with CSB were mixed to assay mixture of 83.3 µl (100 mM Tricine-NaOH (pH 7.5), 10 mM (nucleoside triphosphate (NTP), ADP and AMP), 10 mM $CaCl_2$ and then incubated for 15 min at 25°C (Moustafa *et al.* 2003). The 16.7 µl of 50% (v/v) TCA was added for stopping the reaction and then chilled on ice. Ferrous sulfate-ammonium molybdate reagents (500 µl) was applied to each sample and the absorption was measured at 660 nm (Moustafa *et al.* 2019). Protein content was determined, and bovine serum albumin was applied as a standard.

SDS-PAGE was used to analyze 5 µg of each sample of 20-days old leaves of growing pea (Moustafa *et al.* 2003). Each sample was mixed by 2x sample loading buffer to obtain final concentrations of 2% LDS (Lithium dodecyl sulfate). 0.01 M Tris-HCl (pH 6.8). 20% glycerol, 1% ß-mercaptoethanol, and 0.01% BPB (bromophenol blue), heated for 5 min at 95°C, and separated by SDS-PAGE. Following electrophoresis, the gels were blotted onto PVDF (ImmobilonTM Transfer Membrane, Millipore) membrane and probed with anti-apyrase antibody from rat as the primary antibody and biotinylated anti-rat Ig species-specific whole antibody from sheep (Amersham Pharmacia Biotech) as the secondary antibody. Streptavidin-alkaline Conjugate phosphatase (Amersham Pharmacia Biotech) with BCIP and NBT was used as substrates (Moustafa *et al.* 2003).

For each treatment, the total number of free amino acid was evaluated from dissected leaves of 20-days old planted pea. The 10 ml citrate buffer was used to dissolve 16 mg of ninhydrine solution which was mixed with 10 ml of $SnCL_2$. The prepared reagent (1 ml) was added to the extract of 200 µl leaves and boiled for 20 min. Then 5 ml of diluted solvent (distilled water and 95% ethanol) was applied and kept at 24°C for 15 min. The absorbance was characterized at 570 nm using a spectrophoto-meter. The amino acid contents are expressed in mg g^{-1} dry wt.

The protein content was estimated by using three reagents namely A that prepared by melting 2 g of Na_2CO_3 in 100 ml of 0.1 N NaOH (2 % Na_2CO_3 in 0.1 N NaOH), Reagent B prepared by dissolving 0.5 g $CuSO4.5H_2O$ in 1 % sodium-potassium tartarate. The alkaline reagent solution consists of 50 ml of reagent A and one ml of reagent B. Five ml of the alkaline reagent solution were mixed thoroughly with one ml of the leaves water extract in test tube and kept at 24°C for 10 min, then 0.5 ml of the diluted Foline-Ciocalteau reagent (1:1 v/v) was added to the above mixture and mixed immediately. After 30 min, the absorbance against appropriate blank was evaluated at 750 nm. A calibration curve was constructed using egg albumin and the protein concentration was expressed mg g^{-1} dry wt.

The contents of chlorophylls and carotenoids were determined by using thin-layer chromatography, d. Crude extract of pigments was obtained using acetone: ethanol mixture (3:1) after grinding the leaves using a mortar and pestle with $CaCO_3$. In a mixture of adsorbents on a 20 cm glass plate, chlorophylls and carotenoids were chromatographically isolated and identified by their absorption spectra using spectrophotometer and the content of each pigment was determined according to (Ladygin *et al.* 2004, Pocock *et al.* 2004).

The data were analysed using Microsoft Excel ® and various graphs were obtained. The results were subjected to one-way (ANOVA) analysis using post hoc test by SPSS. *P* value < 0.05 was considered as significant result.

Results and Discussion

Fig. 1 shows the effect of different applications of yeasts extracts on germinating pea (*P. satvium*) against NTP, ADP and AMP. It is apparent that there was no any hydrolytic activity of

all test samples against AMP substrate. In all cases application of the yeast extract in the soil combined with foliar spraying (YE + Soil + Foliar) showed highest analysis activities of adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), thiamine triphosphate (TTP), uridine triphosphate (UTP) and adenosine diphosphate (ADP). The analysis activities of tri and di-nucleotides by applying the yeast extract in the soil only (YE+Soil) was intermediate whereas the foliar spray (YE + Foliar) had the lowest activity. In addition, in all cases CTP potency analysis from the leaves extract of pea found to be higher than other nucleotides. The highest differences of analysis were found between CTP and ATP by 57.87% in case of applying yeast extract in the soil combined with foliar spray (YE + Soil + Foliar) and by 68.80% than untreated control. The least differences were found between CTP and UTP substrate analysis activity by 9.37% for (YE + Soil + Foliar) and by 16.61% than referenced sample. (YE + Soil) caused ATP activity analysis to be more than control by 9.52%, (YE + Foliar) by 3.66% and (YE + Soil + Foliar) by 11.69%. CTP substrate analysis increased by 1.16% for (YE + Foliar), by 4.02% for (YE + Soil) and by 4.81% for (YE + Foliar + Soil) than control. GTP, TTP, UTP and ADP substrate analysis for (YE + Soil) increased between (2.90 to 4.47%), for (YE + Foliar) between (0.13 and 1.20%) and for (YE + Foliar + Soil) between (11.69 and 4.01%) than control. There was statistically significant correlation between the potency of the yeast extracts and substrate analysis in case of (YE + Soil) and (YE + Foliar + Soil) for ATP, (YE + Soil) and (YE + Foliar + Soil) for CTP and (YE + Soil + Foliar) for GTP, UTP and ADP.

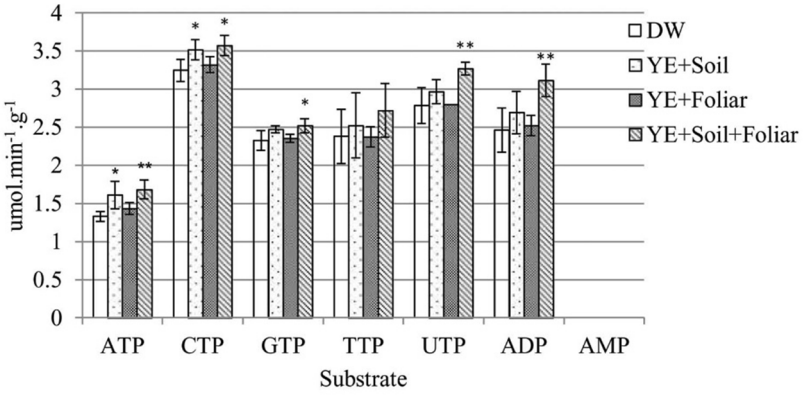

Fig. 1. Substrates analysis activities of grown pea plants in distilled water (DW); yeast extract in the soil (YE + Soil); yeast extract in the foliar part (YE + Foliar); yeast extract in the soil combined with foliar spraying (YE + Soil + Foliar). All analyses are the means of triplicate measurements ± standard deviation. *p <0.05; **p < 0.01.

Densitometry quantification of 49kDa apyrases expression for each treatment in the western blot graphs using Image Master is shown in Fig. 2 (A and B). It is apperant that 49-kDa apyrase was abundant in case of (YE + Foliar + Soil) than (YE + Soil) and little amount for (YE + Foliar). Amount of 49 kDa apyrase in case of (YE + Foliar + Soil), (YE + Soil) and (YE + Foliar) was more than untreated plants by 15.77, 12.05 and 0.86%, respectively. The differences between groups treated with yeast extract and non-treated samples showed that yeast extract applied in the soil combined with foliar spray or in the soil only had a significant effect on physiological activities of germinating pea plants.

Results presented in Figs 3 and 4 showed that amino acids and protein contents were higher in case of application of (YE+ Foliar + Soil) more than (YE + Soil) followed by (YE + Foliar),

whereas seeds treated with DW found to be the lowest. Amount of amino acids in case of using (YE + Foliar + Soil), (YE + Soil) and (YE + Foliar) were found to be more than referenced sample by 40.15, 29.03, and 18.08%, respectively. Amount of protein was found to increase in case using (YE + Foliar + Soil) by 9.13%, for (YE + Soil) by 3.68% and for (YE + Foliar) by 2.85% than referenced samples. Application of yeast extract caused a significant increase ($p < 0.05$) in amino acids content in the germinated pea plant compared to the untreated control.

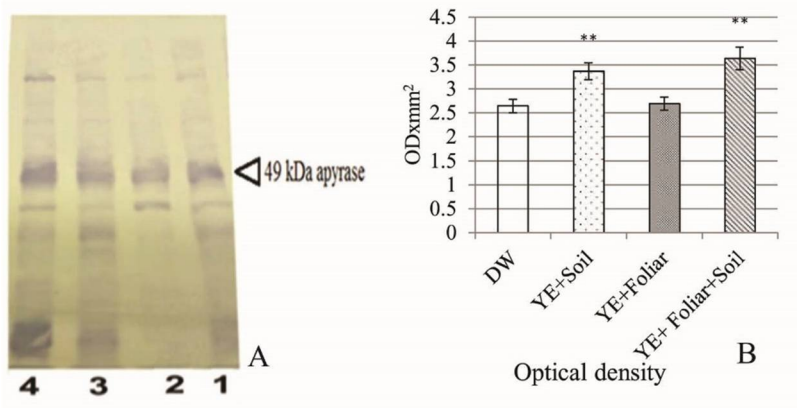

Fig. 2. Panel A,49 kDa apyrase expression of grown pea plants in distilled water (DW), Lane 1; yeast extract in the soil (YE+Soil), Lane 2; yeast extract sprayed in the foliar part (YE+Foliar), Lane 3; yeast extract in the soil + to the foliar spraying (YE+Soil+Foliar), Lane 4. Panel B, Quantification of 49 kDa apyrase. All analyses are the means of triplicate measurements ± standard deviation. *P < 0.05; **p < 0.01.

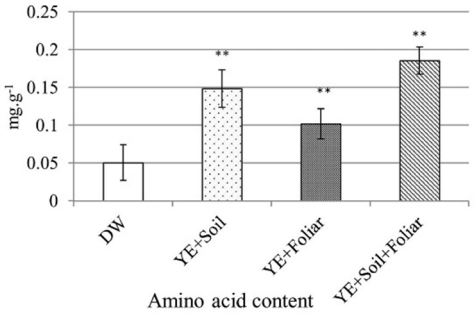

Fig. 3. Amino acids content of grown pea plants. Treatments and statistical details as in Fig. 1.

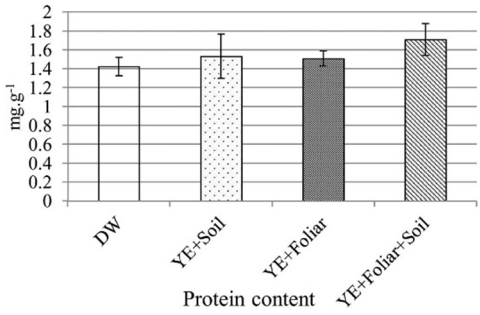

Fig. 4. Protein content of grown pea plants. Treatments and statistical details as in Fig. 1.

The pea plants treated with (YE + Foliar + Soil) were found to possess the highest amount of chlorophyll *a*, *b* and carotenoids (Fig. 5). Chlorophyll *a* increased by 4.31, 1.54 and 7.80% when the plant was treated by (YE + Soil), (YE + Foliar) and (YE + Foliar + Soil), respectively. The same trend was found in Chlorophyll *b*, carotenoids and the total amount that increased in a range of between (2.41 and 13.10%) than control (Fig. 5). The only significant differences ($p < 0.05$) between groups treated with yeast extract and referenced samples was found in the amount chlorophyll *a* for (YE + Foliar + Soil) and in the carotenoids for (YE + Foliar + Soil) and (YE + Soil).

It is well known that apyrase is a member of the ATPase family of E-type that hydrolyzes both π- and β-phosphate on adenosine triphosphate or adenosine diphosphate but not nucleoside monophosphates. ATPase and ADPase activities were found as a measuring tool of plant metabolic activity as it was found to play a vital role in many processes during germination and in plant development (Palmgren 2001). The presence of ATP and/or ADP decomposing activity on the surface of several cell types has been recognized for many decades. In addition, the legume-specific apyrases like DbLNP from *Dolichos biflorus* and GS52 from *Glycine soja* which located in the cell membrane were proven to have a role in the formation of root nodulations (Kalsi and Etzler 2000). The present results showed that yeast extract either in the soil or sprayed in the foliar

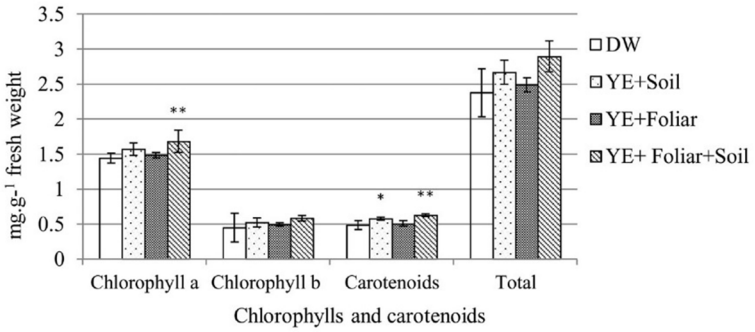

Fig. 5. Chlorophyll *a*, chlorophyll *b*, carotenoids and total of photosynthetic pigments of grown pea plants. Treatments and statistical details as in Fig. 1.

parts could enhance the hydrolysis of NTP and ADP that yielding inorganic phosphate. It was found that extracts from various stages of germination pea plant and from different organs had maximal activity against cytidine triphosphate and least against adenosine triphosphate, with activity against guanosine triphosphate, thiamine triphosphate, uridine triphosphate and adenosine diphosphate found to be intermediate and no hydrolytic activity against adenosine monophosphate (Moustafa *et al.* 2003). In consistent with study which proven that an application of yeast was found to play various physiological role to the (*Sorghum bicolor* L.) and (*Zea mays* L.) plants in elevating the stress effect of sea water and could increase fresh and dry weights of shoot and root systems, chlorophyll contents, hairs on the leaves, stomatal density and furrows numbers in roots (Moustafa and Al-Shehri 2020). Lonhienne *et al.* (2014) reported that yeast also can increase the root/shoot ratio in (*S. officinarum* x *spontaneum*) and (*S. lycopersicum*) and caused morphological changes including increased the tillering numbers of sugar cane and increased biomass of tomato plants. Increased analysis rate in the existence of yeast extract support that adenosine 5′-triphosphate (ATP), and also other nucleoside triphosphates, are energy-supplying agents for various reactions within cells, both in animal and plant species (Tripathi and Tanaka 2018). The strong positive correlations between expression of 49 kDa apyrase and potency analysis of various nucleotides indicate that apyrase could be key signaling molecules in certain plant processes, such as cellular differentiation, photosynthetic activity and biotic and abiotic protection (Moustafa *et al.* 2003, Moustafa *et al.* 2019). Pietrowska-Borek *et al.* (2020) reported that the hypothesized apyrase (APY) found on the plasma membrane can reduce the extracellular adenosine triphosphate concentration in the extracellular matrix directly. It was also reported that the suppression of expression the two apyrases APY1 and APY2 caused the *Arabidopsis thaliana* plant to be dwarfism, eATP over-accumulation and impaired polar auxin transport (Liu *et al.* 2012).

In the present study, the potency of the three-tested applications as a stimulator to the amino acids content, protein content and the amount photosynthetic pigments were found to be (YE+Foliar+Soil) > (YE+Soil) > (YE+Foliar) > DW. Again, the strong correlation between the efficiency of the rate of apyrase expression, substrate analyses activity and the rest of the tested biomarkers are probably due to the active components found in the yeast extract. This improvement in these characteristics could be attributed to the capability of yeast to increase the production of plant growth hormones, as auxins, gibberellins and cytokinins, which act to improve various metabolic activities of plant (Kahlel 2015). Cytokinins content, vitamin B_5 and many other minerals present in yeast extract were found to play an important role in the orientation and transfer of metabolites from leaves to active organs (Al-Hawezy and Ibrahim 2018; Hussain *et al.* 2012). In addition, several researchers showed the importance of yeast extract application on vegetative growth parts and for pod yield of pea plants in case of foliar supplement (Mahmoud *et al.* 2013). Thus the findings of the present investigations are in consistent with previous studies which confirmed the importance of yeast extract in plant development in a variety of physiological processes. However, future research is warranted to investigate more or less concentration from yeast extract to be applied to the pea plants to get the highest values of the above mentioned plant growth characters. In conclusion, it may be inferred that supplying pea plant with yeast extract to the soil along with foliar spray enhanced 49 kDa gene apyrase, NTP, ADP substrate analysis, amino acids content, protein content and photosynthetic pigments. These findings also indicate that yeast extract would be a useful potential bio-fertilizer in improving crop growth and nutrient supply.

Acknowledgements

The author are thankful to the Deanship of Scientific Research, King Khalid University, Saudi Arabia, for funding under grant number (G.R.P. 65-41).

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(*Manuscript received on 26 May 2021; revised on 7 April 2022)*