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Potentiality of Some Yeast Species for Promotion of Growth and Productivity of Soybean Plants (*Glycine max*. L.)

Mamdouh S. Haridy, Gaber K. Abd El–Baki, Esraa N. Fawzy*

Botany and Microbiology Department, Faculty of Science, Minia University, 61519, El-Minia, Egypt.

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Abstract

A total of seven yeast strains were isolated from rhizosphere soils of soybean plants cultivated at different localities of El-Minia Governorate. Strains were tested for their capability to promote growth of soybean plant in pot experiments containing sterilized soil. Results showed that all the seven tested yeast strains could promote soybean plant growth but with different degrees compared with control. Only two strains of yeast (***Saccharomyces cerevisiae*** and ***Torulaspora delbrueckii***) had high superiority for promotion of soybean growth, therefore, these two yeast species were selected for further studies in field trials.

Results of field trials revealed that ***Saccharomyces cerevisiae*** and ***Torulaspora delbrueckii*** affected all growth parameters and yield traits of soybean plants significantly. After 30 days from seed germination, they increased plant root and shoot growth, their fresh and dry matter, leaf surrounding, photosynthetic pigments (Chlorophyll a, b, and carotenoids). After 50 days, number of nodules per plant root and its dry weights were also increased. Also, results revealed that, treatment with ***S. cerevisiae*** and ***T. delbrueckii*** increased plant height, number of branches, number of fruit clusters, number of pods, number of seeds, weight of seeds, hundred seed weights, and seed production/feddan.

Of interest was the seed quality of treated plants, its total, soluble and insoluble proteins, oil and Tannic acid contents. *In vitro* indol-3-acetic acid and polyamine production in synthetic medium were detected.

In conclusion, yeast species of ***Saccharomyces cerevisiae*** and ***Torulaspora delbrueckii*** represent promising candidates for successful biofertilizers and could be used for sustainable agriculture in the future.

Keywords

Saccharomyces, Torulaspora, Soybean, growth promotion, seed quality

Introduction

Is well known that microbial activity in the rhizosphere affected plant growth and its productivity [1-6].Therefore, it is not astonishing that some yeast species isolated from the rhizosphere and the bulk soil have capability to promote plant growth and its productivity [2, 4, 5, 7, 8]. These plant growth promoting yeasts (PGPYs) are belong to some yeast genera such as ***Candida, Rhodotorula, Trichosporon, Saccharomyces, Cryptococcus, Rhodosporidium, Sporobolomyces, Williopsis*** and ***Yarrowia*** [2-5 ,7, 9-12] . Depending on yeast taxa, the mechanisms of plant growth promotion are clearly varied. These mechanisms include pathogen inhibition [2, 5, 13], phytohormone production [4, 7, 14-16], phosphate solubilisation [7, 17-23], N and S oxidation [17], Siderophore production [13], polyamine production [11] and stimulation of mycorrhizal-root colonization [18, 20]. Soybean represents one of the largest sources of vegetable oil and of animal protein feed in the world [24]. It has the highest protein content (40–42%) of all other food crops and ranks second only to ground nut with respect to the oil content (18–22%) among food legumes [25]. It is also used for aquaculture and biofuel, as well as a protein source for the human diet [26]. Obesity and muscle fatigue can be prevented by soybean protein [27]. The major soybean producers in the world are the USA, Brazil, Argentina, China and India with more than 92% of the world's soybean production [26, 28]. Since the twentieth century, soybean has also been produced in Africa [29]. In Africa, soybean could possibly become a major crop owing to its many uses as a food, feed and in industry as well as its ability to undertake symbiotic nitrogen fixation making it with a great advantage over cereal crops [30]. Soybean represents a relatively new crop introduced into Egyptian agriculture in order to reduce the food deficiency gap, as its seeds contain high ratio of protein and oil which reach up to 40 and 20% respectively [31]. In Egypt, soybean acreage has dramatically decreased during the last twenty years from about 100 000 fed. In 1991 to about 9000 fed. In 2011 season [32] which due to competition with other summer crops, increase in production cost, reduction of net profit per unit area and problem in marketing process. Consequently, the total soybean production become below the requirement of consumption. Increasing soybean production can be achieved through either growing the new high yielding and insect resistant soybean genotypes using biofertilizers as an option for its yield promotion or increasing soybean production areas. Therefore, the current study aimed at using some yeast species isolated from soybean rhizosphere soils to promote soybean plant growth and its productivity. So that, objectives of the current work were:

Isolation and identification of some yeast strains from rhizosphere soils of soybean plants grown in farmland at different localities in El-Minia Governorate.

2- Determination of ability of the isolated yeasts to promote soybean plant growth, enhance yield and improve seed quality.

*** Correspondence: Esraa N. Fawzy***

Tel.: *+2* ***01092646562; Fax:***

Email Address: nasseresraa218@gmail.com

3- Study some of yeast mechanisms that are responsible for soybean plant growth promotion.

2. Materials and Methods

2.1. Collection of rhizosphere soil samples, isolation and identification of its yeast contents:

A total of 25 soil samples were collected from rhizosphere areas of soybean plants cultivated at several localities (Abo-Korkas, Mallawy and El-Minia) in El-Minia Governorate, Egypt. Samples were kept in sterile Erlenmeyer flasks and transferred directly to the laboratory. Soil samples (5-10 gm) were mixed with 50 ml sterile distilled water, vigorously shaken to prepare a serial dilutions for inoculating Petri-dishes containing YM agar medium [33]. Sodium propionate (0.2%) was added to the solid medium to inhibit growth of filamentous fungi. Plates were incubated at 25ºC for 3-5 days. Yeast colonies were examined microscopically, picked, purified and maintained on YM agar slants. Identification of the isolated yeast strains was carried out using the procedures described by [34], and reference strains (CBS) provided by Centraalbureau voor Schimmelcultures, Delft, the Netherlands.

2.2. Coating of soybean seeds with fresh yeast cells of each of *Saccharomyces cerevisiae, Torulaspora delbrueckii and Kluyveromyces marxianus:*

Yeast strains of each of the obtained yeast species were selected on the basis of its preliminary testing for plant growth promotion. Fresh yeast cells were taken by inoculating 250 ml Erlenmeyer flasks containing 50 ml YM broth. Cultures were incubated at 25ºC on rotary shaker adjusted to 200 rpm for 2 days. Culture media were centrifuged at 6000 rpm for 10 minutes and supernatant was decanted and yeast cream mass was washed twice with sterile distilled water to remove any remains of culture medium. Eventually, fresh yeast cells were suspended in sterile distilled water and their counts were adjusted at concentration1.2-2 x 10^7 cfu/ml using haemocytometer slide under light microscope. Yeast cell suspension was mixed individually with appropriate amount of carboxymethylcellulose (CMC). On the other hand, soybean seeds were surface sterilized by soaking in the solution of sodium hypochlorite (2% W/V) for 2 min and washed several times with sterile distilled water. The Sterilized soybean seeds were added to the previously prepared mixture (yeast-CMC) and stirred thoroughly (for one hr). Seeds were removed, spread in sterile opened Petri-dishes and allowed to dry overnight in a laminar flow cabinet at room temperature.

2.3. Petri-dish experiments:

Filter papers (Whatman No 1) were placed in Petri-dishes (9 cm diameter) and sterilized. A total of 10 surface sterilized soybean seeds were spread in each Petri-dish; un-coated seeds as (control) and the previously prepared coated soybean seeds with yeast-CMC of each obtained and tested yeast species were performed, then seeds were irrigated with equal amounts of sterilized water and incubated at room temperature for 12days. Experiments were performed in triplicates and repeated twice. The following traits of seed germination and seedlings were determined: time of beginning of seed germination, percentage of seed germination, seedling length and number of root hairs per seedling.

2.4. Pot experiments:

Sterile plastic pots (16 cm diameter,13cm height) containing 350gm sterile soil collected from Botanical garden of Botany and Microbiology Department, Faculty of Science, Minia University were prepared. Treatments in triplicates were carried out as follows:

a - Non coated seeds were sown in sterile soil (control).
b - Seeds coated with fresh yeast cells of ***S. cerevisiae*** were sown in sterile soil.
c - Seeds coated with fresh yeast cells of ***T. delbrueckii*** were sown in sterile soil.
d - Seeds coated with fresh yeast cells of ***K.marxianus*** were sown in sterile soil.

Soils were irrigated with equal amounts of distilled water and placed at ambient temperature in Botanical garden. Seed germination percentage and seedling growth parameters including root and shoot lengths, their fresh and dry weights, leaf surrounding and its photosynthetic pigments (chlorophyll a, b and carotenoid pigments) were determined after 15 days of seed sowing and the experiment was repeated twice. On the basis of obtained results from pot experiments, ***K. marxianus*** was excluded from field trials as it was the least soybean plant growth promoter in pot experiment.

2.5. Field trials:

Field experiments were performed at Experimental Station, Ministerium for Agriculture, Mallawy, El-Minia Governorate, Egypt during 2020 summer season on a sandy silt clay soil. Soil had the following physical and chemical characteristics: Sand % 7.90; salt% 54.50 ; clay% 37.60; pH 8.2, E.C.(ds/m 1.35); organic matter % 1.18; available N 20.35 ppm; P 8.15 ppm; K 183 ppm; soluble cations: Ca^{+2}, 6.25; Mg^{+},0.76; K^{+},0.20; Na^{+}, 2.85; soluble anions : CO_3^{-2} , 0.0; HCO_3^{-}, 2.05 ; Cl^{-}, 2.25 and SO_4^{-2}, 5.85. The experiments were conducted in a complete randomized plot design, where the plot size was 4m in length x 1.8 m in width in 3 replicates. Each plot consisted of 3 lines with 4 m in length and 60 cm in width. Plants per hall between 20 cm apart and one ridge. Three treatments were included in the experiment and were arranged in a complete randomized plot design. The following treatments were used:

1- Un-coated seeds were sown in the soil
2- Coated seeds with fresh yeast cells of ***Saccharomyces cerevisiae*** were sown in the soil.
3- Coated seeds with fresh yeast cells of ***Torulaspora delbrueckii*** were sown in the soil.

In soil preparation, all plots received the recommended dose of phosphorus (15.5 Kg P_2O_5/ feddan) in form of Calcium super phosphate 15.5% and ammonium nitrate (50 Kg/feddan) in form of ammonium nitrate 33.5% N, once after the first irrigation. The normal agriculture practices commonly used for soybean were followed. Regarding determination of vegetative growth parameters, 6 plants from each plot were picked up randomly after 30 days of sowing as representative samples for measuring the following characteristics: root and shoot lengths, their fresh and dry weights, leaf surrounding and photosynthetic pigments (chlorophyll a, b and carotenoid pigments), which were determined using the spectrophotometric method recommended by **Metzner** *et al*. [35]. The plants were grown for 120 days under field conditions. Water was supplied regularly as needed.

Nodulation was determined after 50 days after planting by counting the number of nodules on 6 plant roots selected randomly from each plots. Nodules were dried at 60ºC for 2

days and then its dry weight was estimated. At harvesting time (120 days), plant heights and yield parameters of 6 plants chosen from each plot and the following yield traits were estimated: number of branches/plant, number of fruit clusters/plant, number of pods/plant, number of seeds/plant, weight of seeds/plant, 100 seed weight, seed yield (Kg/feddan). In regard to seed quality, oil percentage, total protein, soluble as well as insoluble protein and total tannins of the treated and non-treated seeds were determined. Oil percentage of seeds was estimated using procedure described by **Bokhari** *et al.* [36], whilst total proteins were determined according to method of **Beljkas** *et al.* [37]. Estimation of soluble protein was carried out according to the method adopted by **Lowry** *et al.* [38]. Total tannins were determined by procedures of **Linskens and Jackson** [39].

2.6. Quantification of indole-3-acetic acid using Salkowski reagent:

Potentialities of the tested isolated yeasts to produce indole-3-acetic acid were carried out according to the method described by **Gordon and Weber** [40].Yeast strains were grown in test tubes in YPD medium with or without 0.1% (W/V) L-tryptophan and incubated in the dark on rotary shaker at 30ºC and 150 rpm for 5 days, then one milliliter of the culture was pelleted by centrifuging at 3000 rpm for 5 min and take 0.5 ml of the supernatant to be mixed with 1 ml of Salkowski reagent (2 ml of 0.5 M iron (III) chloride and 98 ml of 35% perchloric acid). After 30 min pink color development was measured using a UV-visible spectrophotometer (R.S.1500) at 530 nm. A calibration curve using pure indole-3-acetic acid was established for calculating indole-3-acetic acid concentration.

2.7. Assay of inorganic phosphate solubilizing ability:

Yeast strains were grown in sterilized liquid medium described by **Nautiyal** [41] at 30ºC for 7 days under shaking at 150 rpm . The sterilized un inoculated medium served as a control. Aliquot (10 ml) of each culture and control medium was centrifuged at 8000 rpm for 15 min. The clear supernatant was used in determining the amount of phosphorous released into the medium. The pH of the culture medium was also recorded with a pH meter equipped with glass electrode. Phosphorus availability was determined using phospho-molybdate blue color method [42].

2.8. Polyamine production:

Capabilities of Polyamine production by the two of the tested yeast species were carried out using Long Ashton Decarboxylase (LAD) broth described by **Cloete** *et al.* [11]. Long Ashton decarboxylase broth (pH 5.5) was supplemented with 2gm/L L-arginine monohydrochloride (Sigma) and 0.02 g/L phenol red (Sigma) as pH dye indicator. Broth without supplementing with L-arginine was used as control. Broth was inoculated with fresh yeast cell suspension at 10^6 cfu/ml and incubated at 28ºC on rotary shaker at 150 rpm for 4 days. The appearance of pink color in the broth was indicative of arginine decarboxylation activity and potential polyamine production. The intensity of pink color can be determined as readings at optical density 530 nm using UV-visible Spectrophotometer (RS1500).

2.9. Statistical analysis:

All data recorded in the season were subjected to proper statistical analysis according to procedures outlined by **Steel and Torrie** [43]. The differences among treatment means were compared using Least Significant Differences test (L.S.D) at 5% level of probability.

3. Results and Discussion

3.1. Isolation and identification of yeast strains from rhizosphere soil of soybean plants:

Rhizosphere soil of plants represents a good shelter to huge diversity of microorganisms including bacteria, filamentous fungi and yeasts. Yeast populations are higher in the rhizosphere soils compared to the bulk soils [7, 11, 44, 45]. Therefore, rhizosphere soil of soybean plant were screened to isolate some yeast strains in order to use these yeasts for promotion of soybean plant growth, enhancement its yield and improvement its seed quality. A total of 7 yeast strains were isolated from rhizosphere soil of soybean plants cultivated in different localities in El-Minia Governorate. On the basis of 24 morphological and physiological properties as well as using reference strains provided from culture collection of Centraalbureau voor Schimmel cultures (CBS), Delft, the Netherlands, yeast strains were assigned to 3 yeast species, ***Saccharomyces cerevisiae*** (2 strains), ***Kluyveromyces marxianus*** (2 strains) and ***Torulaspora delbrueckii*** (3 strains) **(Table 1)**. Previously, Haridy [44] isolated ***Torulaspora delbrueckii*** from rhizosphere soils of potato and cabbage plants, [7] isolated ***Kluyveromyces marxianus***, ***Saccharomyces cerevisiae*** and ***Torulaspora delbrueckii*** from rhizosphere soils of fennel plants.

3.2. Effects of soybean seed coating with fresh yeast cells of the isolated yeast species on seed germination and seedling growth parameters in Petri-dish experiments:

Promotion of plant growth using yeasts was recorded by many investigators [2, 4, 7, 11, 12, 44, 46-48]. The obtained results of Petri-dish experiments revealed that soybean seeds coated with fresh yeast cells of each of ***Saccharomyces cerevisiae***, ***Torulaspora delbrueckii*** and ***Kluyveromyces marxiauns*** at concentration 1.2 – 2 x 10^7 cfu/ml reduced the required time for beginning of seed germination and increased percentage of its germination compared to non-coated seeds. The coated seeds by ***Saccharomyces cerevisiae***, ***Torulaspora delbrueckii*** and ***Kluyveromyces marxiauns*** germinated earlier by 24, 36 and 36 hrs prior to uncoated seeds, respectively. Germination percentage of non-coated seeds was 50%, whilst percentage of germination of the treated coated seeds with yeast cells of ***Saccharomyces cerevisiae***, ***Torulaspore delbrueckii*** and ***Kluyveromyces marxiauns*** was 90, 70 and 63%, respectively **(Table 2)**. Both of seedling length and number of root hairs formed per seedling were also affected by treating seeds with fresh yeast cells in comparison to untreated seeds. Treatment of seed with ***Saccharomyces cerevisiae***, ***Torulaspora delbrueckii*** and ***Kluyveromyces marxiauns*** increased seedling length by 58.1, 91.9 and 43.5%, consecutively, while number of root hairs increased by 66.7, 166,7 and 50%, respectively **(Table 2, plate 1)**. Sun *et al*. [15] showed that co cultivation of arabidopsis plants with yeasts enhances formation of lateral

root and root hairs. Lateral roots extend horizontally from the primary root to anchor the plant securely into the soil. Root hairs increase the surface area of a root and play critical roles in the uptake of water and nutrients which implied a beneficial effect of yeast inoculation on plant growth and development. Bab'eva and Belyanin [46] pointed out that cabbage seed germination was stimulated by soaking cabbage seeds in culture filtrates of nine strains of ***Torulopsis*** species. El-Mehalawy *et al*. [49] reported that two yeast species of ***Saccharomyces unisporus*** and ***Candida steatolytica*** that isolated from kidney bean rhizosphere significantly increased percentage of seed germination when they were added as biological control agents against ***Fusarium oxysporum***. Moreover, germination rates of different seeds and grains of pea, bean, barley and maize were enhanced by using different yeast dilutions [7, 50]. In addition, germination of fennel seeds coated with fresh yeast cells of ***Saccharomyces cerevisiae*** at conc. 1.8 x 10^9 cfu/ml increased by 24.4% compared to non-coated seeds**.**

3.3. Effect of yeast cell application of the isolated species on soybean seedling growth in pot experiments:

Promotion of plant growth using yeasts was recorded by many investigators [2, 4, 7, 11, 12, 44, 46-48]. The obtained results of Petri-dish experiments revealed that soybean seeds coated with fresh yeast cells of each of ***Saccharomyces cerevisiae***, ***Torulaspora delbrueckii*** and ***Kluyveromyces marxiauns*** at concentration 1.2 – 2 x 10^7 cfu/ml reduced the required time for beginning of seed germination and increased percentage of its germination compared to non-coated seeds. The coated seeds by ***Saccharomyces cerevisiae***, ***Torulaspora delbrueckii*** and ***Kluyveromyces marxiauns*** germinated earlier by 24, 36 and 36 hrs prior to uncoated seeds, respectively. Germination percentage of non-coated seeds was 50%, whilst percentage of germination of the treated coated seeds with yeast cells of ***Saccharomyces cerevisiae***, ***Torulaspore delbrueckii*** and ***Kluyveromyces marxiauns*** was 90, 70 and 63%, respectively **(Table 2)**. Both of seedling length and number of root hairs formed per seedling were also affected by treating seeds with fresh yeast cells in comparison to untreated seeds. Treatment of seed with ***Saccharomyces cerevisiae*** ,***Torulaspora delbrueckii*** and ***Kluyveromyces marxiauns*** increased seedling length by 58.1, 91.9 and 43.5%, consecutively, while number of root hairs increased by 66.7, 166,7 and 50%, respectively **(Table 2, plate 1)**. Sun *et al*. [15] showed that co cultivation of arabidopsis plants with yeasts enhances formation of lateral root and root hairs. Lateral roots extend horizontally from the primary root to anchor the plant securely into the soil. Root hairs increase the surface area of a root and play critical roles in the uptake of water and nutrients which implied a beneficial effect of yeast inoculation on plant growth and development. Bab'eva and Belyanin [46] pointed out that cabbage seed germination was stimulated by soaking cabbage seeds in culture filtrates of nine strains of ***Torulopsis*** species. El-Mehalawy *et al*. [49] reported that two yeast species of ***Saccharomyces unisporus*** and ***Candida steatolytica*** that isolated from kidney bean rhizosphere significantly increased percentage of seed germination when they were added as biological control agents against ***Fusarium oxysporum***. Moreover, germination rates of different seeds and grains of pea, bean, barley and maize were enhanced by using different yeast dilutions [7, 50]. In addition, germination of fennel seeds coated with fresh yeast cells of ***Saccharomyces cerevisiae*** at conc. 1.8 x 10^9 cfu/ml increased by 24.4% compared to non-coated seeds**.**

3.3. Effect of yeast cell application of the isolated species on soybean seedling growth in pot experiments:

The results obtained from pot experiments after 15 days of seed germination showed that there were significant increases in all tested parameters of plant growth for the treated seeds in comparison to untreated seeds. Root and shoot length of seeds singly coated with ***S. cerevisiae***, ***T. delbrueckii*** and ***K. marxianus*** increased by 11.1, 28.3, 6.1; 13.9, 22.5, 6.5%, respectively, whilst their fresh and dry weights increased by 33.3, 29,2, 27.1; 29,2, 24.9, 14.5; 9.5, 11.3, 5.5; 15.0, 18.3, 13.4%, respectively **(Table 3, Figure 1)**. Both of leaf surrounding and its photosynthetic pigments were positively influenced. Leaf surrounding increased by 42.5, 36.3 and 25%, respectively, when seeds were treated with ***S. cerevisiae***, ***T. delbrueckii*** and ***K. marxianus***, while chlorophyll a,b and carotenoids increased by 49.2, 43.1, 24.6; 50.0, 10.7, 25.0; 44.4, 55.6 and 16.7%, consecutively **(Table 3, Figure 1)**. It was clear from the results found in **(Table 2 & 3**) that ***Kluyveromyces marxiauns*** was the least tested cells of yeast promoter for soybean plant growth, therefore it was excluded from further studies in the field experiments.

Table 1: Physiological and morphological properties of the isolated yeast strains and reference strains.

Tested yeast species	Fermentation sources						Assimilation sources											growth on glucose		growth temper-ature		Formation Products			
	total no. of tested strains (10)	glucose	galactose	sucrose	maltose	lactose	raffinose	galactose	sorbose	xylose	ribose	arabinose	rhamnose	sucrose	maltose	lactose	raffinose	50 %	60 %	37 °C	42 °C	true mycelium	pseudomycelium	ascospores	Pellicles
S. cerevisiae	**2**	+	+	+	-	-	+	+	-	-	-	-	-	+	+	-	+	+	-	+	+	-	-	+	-
S*. cerevisiae	**1**	+	+	-	-	-	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+	-
T. delbrueckii	**3**	+	+	+	-	-	+	+	-	-	-	-	-	+	-	-	-	+	-	+	-	-	-	+	-
T*. delbrueckii	**1**	+	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	+	-
K. marxianus	**2**	+	+	-	-	+	+	+	+	+	+	-	-	+	-	+	+	+	-	+	-	-	+	+	+
K*. marxianus	**1**	+	+	-	-	+	+	+	+	+	-	-	-	+	-	+	+	+	-	+	+	-	+	+	+

S*. = *Saccharomyces cerevisiae* (a reference strain CBS 2858)
T*. = *Torulaspora delbrueckii* (a reference strain CBS 4510)
K*. = *Kluyveromyces marxianus* (a reference strain CBS 1574)
+ = positive - = negative

Table 2 : Effect of seed coating with fresh yeast cells of the tested yeast species on time of seed germination, number of germinating seeds, their seedling length and their number of root hairs at room temperature after 12 days (Petri- dish experiments).

Treatment	Time of beginning seed germination (hr)	number of germinated seeds (out of 10 seeds)	seedling length (cm)	number of root hairs/ seedling
untreated seeds	60	5*	6.2	6
seeds treated with *S. cerevisiae*	24	9	9.8	10
Effectiveness %	- 60	+ 80	+ 58.1	+ 66.7
seeds treated with *T. delbrueckii*	36	7	11.9	16
Effectiveness %	- 40	+ 40	+ 91.9	+ 166.7
seeds treated with *K. marxianus*	36	6.3	8.9	9
Effectiveness %	- 40	+ 26	+ 43.5	+ 50

***S.* = *Saccharomyces* *T.* = *Torulaspora* *K.* = *Kluyveromyces* * = average of 3 plants**

Table 3: Effect of seed coating with yeast cells of the tested yeast species on root and shoot length, fresh and dry weights, surrounding of leaf and photosynthetic pigments of seedlings at ambient temperature after 15 days from seed germination (pot experiment).

Treatment	length(cm)		fresh weight (mg/plant)		dry weight (mg/plant)		surrounding of leaf (cm)	photosynthetic pigments (mg/gm dry weight)		
	root	shoot	root	shoot	root	shoot		Chl.a	Chl.b	Carot
untreated seeds	9.9*	23.1	144.0	1184.5	39.8	121.7	8.0	6.5	2.8	1.8
seeds treated with *S. cerevisiae*	11	26.3	192	1530	43.6	140	11.4	9.7	4.2	2.6
Effectiveness %	11.1	13.9	33.3	29.2	9.5	15.0	42.5	49.2	50.0	44.4
seeds treated with *T.delbrueckii*	12.7	28.3	186	1480	44.3	144	10.9	9.3	3.1	2.8
Effectiveness %	28.3	22.5	29.2	24.9	11.3	18.3	36.3	43.1	10.7	55.6
seeds treated with *K. marxianus*	10.5	24.6	183	1356	42	138	10	8.1	3.5	2.1
Effectiveness %	6.1	6.5	27.1	14.5	5.5	13.4	25.0	24.6	25.0	16.7
L.S.D at (0.05)	1.23	1.44	14.40	142.91	3.87	12.75	1.33	0.78	0.74	0.83

*** = average of 6 plants (except for photosynthetic pigments only 3 plants)**

Chl . = Chlorophyll T. = *Torulaspora* K. = *Kluyveromyces* S. = *Saccharomyces* Carot. = Carotenoids

A **B** **C**

Plate 1: Germenation of untreated soybean seeds (left arrows) compared to seeds treated with fresh yeast cells (right arrows) of the tested yeast species *Saccharomyces cerevisiae* (A), *Torulaspora delbrueckii* (B) and *Kluyveromyces marxianus* (C) at conc. 1.2 – 2.0 × 10^7 cfu/ml at room temperature after 12 days from irrigation of seeds with water (Petri –dish experiments).

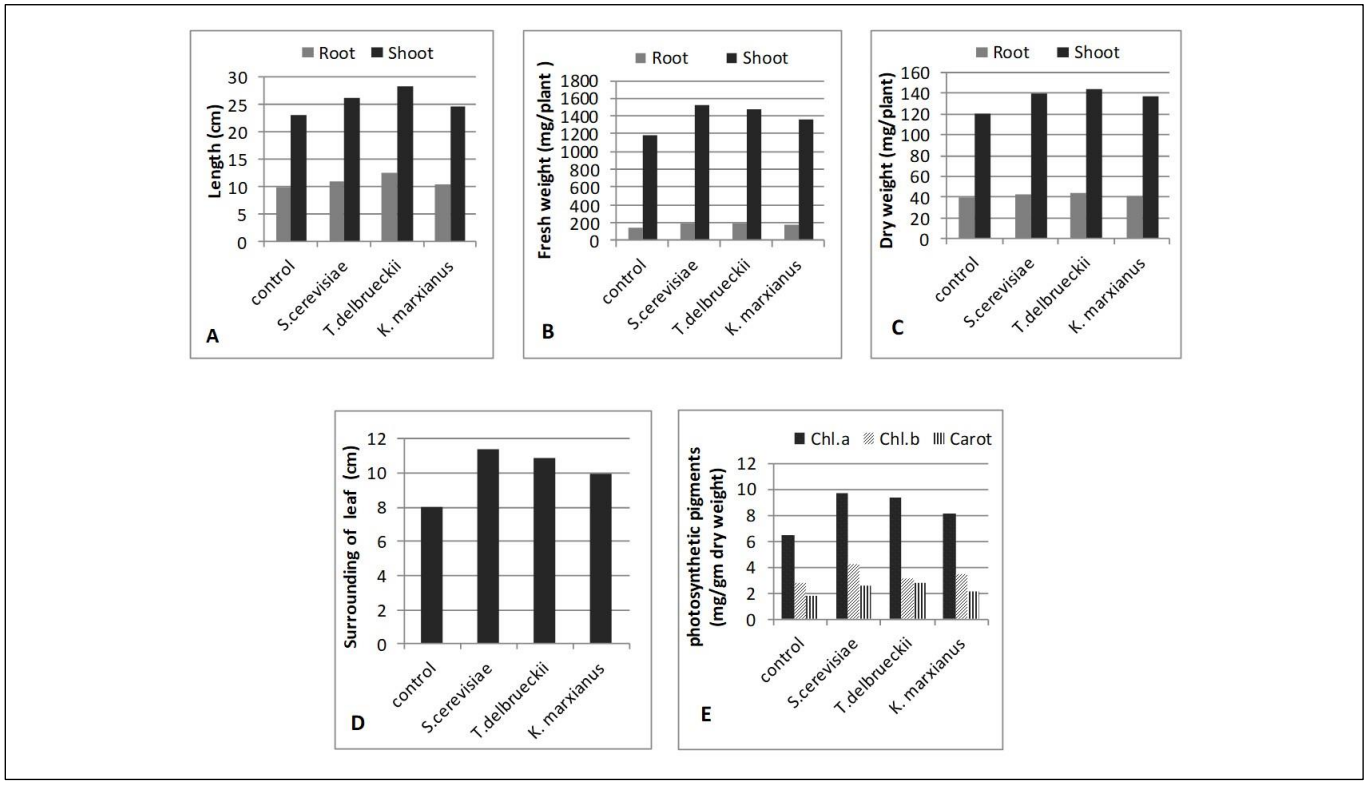

Figure 1: Effect of seed coating with the tested yeast cells on root and shoot length(A), their fresh weights(B), their dry weights (C), surrounding of leaf (D)and its photosynthetic pigments of seedlings (E) at ambient temperature after 15 days from seed germination (pot experiment).

S. = *Saccharomyces* T. = *Torulaspora* K. = *Kluyveromyces* Chl . = Chlorophyll Carot. = Carotenoids

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3.4. Effect of the tested yeast species on promotion of soybean plant growth, enhancement of its yield and improvement its seed quality in field trials:

Results of field in **(Tables 4** and **5)** revealed the similar behavior toward soybean plant growth promotion as well as results of pot experiments except for dry weights of roots and shoots. Treatments of soybean seeds with yeast cells of ***S. cerevisiae*** had higher superiority on plant growth promotion compared to the treatment with ***T. delbrueckii***, whereas, dry weight of root and shoot of ***S. cerevisiae*** - treated plants increased by 86 and 99.2% , while dry weight of root and shoot increased by 56 and 65.3% in seed treatments with ***T. delbrueckii*** **(Table 4, Figure 2)**. Morsy *et al*. [12] reported that the all growth parameters of soybean were significantly enhanced due to application of yeasts, especially ***S. cerevisiae*** [51] proposed that a tryptophan independent pathway for indole-3-acetic acid (IAA) synthesis exists in baking yeast ***Saccharomyces cerevisiae*.** This pathway may also play a major role in the promotion of soybean plant growth in this investigation as indicated by obtained results of pot and field experiments **(Tables 3** and **4)** . Sole treatment of soybean seeds with fresh yeast cells of the two tested yeast species had a considerable effect on the nodulation of soybean plant roots. Treatment of soybean seeds with fresh yeast cells of ***Saccharomyces cerevisiae*** increased number of nodules per plant and its dry weight by 67.7 and 50.2 %, respectively, while ***Torulaspora delbrueckii***-treated soybean seeds showed increases of both nodule number and nodule dry weight by 43.9 and 29% consecutively, after growth for 50 days in the field trials **(Table 5).** Singh *et al*. [9] found that inoculation of legumes with ***Saccharomyces cerevisiae*** increased nodulation as well as arbusculer mycorrhiza fungal colonization. Morsy *etal*. [12] reported that after growth for 75 days, soybean plants inoculated with ***S. cerevisiae*** +3% humic acid + ***Bradyrhizobium japonicum*** 110 give higher nodule number, nodule dry weight and nitrogenase activity. Mohamed and Metwally [52] studied the effect of combined inoculation of *Rhizobium* with soil yeasts on nodulation, growth and yield of common bean under field condition, and the obtained results showed highest improvement of bean nodulation in case of ***Saccharomyces cerevisiae*** or ***S. exogenous*** of the tested yeast species. Mohamed [53] pointed out that ***S. cerevisiae*** improved nodulation of faba bean roots and it was more effective than ***Candida sake***. Synergism between ***S. cerevisiae*** and isolated ***Rhizobium leguminosarum*** induces the nodulation and productivity of ***Vicia faba*** [54].

Flowering of soybean plants were induced by application of fresh yeast cells of the tested yeast species. Yeast species of ***S. cerevisiae*** and ***T. delbrueckii*** induced early flowering of soybean plants 9 and 5 days prior to untreated plants **(Plate 2)**. Wijayanti *et al*. [55] showed that indole-3-acetic acid may be involved in the regulation of early flowering process in the apex of pharbitis nil. Cheng and Zhao [56] reported that auxin play an important role in flower differentiation and development. Auxin is necessary for initiation of floral primordia and disruption of auxin biosynthesis, polar auxin transport or auxin signaling leads to the failure of flower formation. Auxin also plays an essential role in specifying the number and identity of floral organs [56].

3.5. Effects of seed coating with fresh yeast cells of S. cerevisiae and T. delbrueckii on soybean yield traits (field experiments):

Results of the effect of seed coating with fresh yeast cells of each of the two tested yeast species (***S. cerevisiae*** **and** ***T. delbrueckii*****)** on plant height and yield parameters under field conditions at the time of harvest were shown in **(Table 6)**. Treatments of soybean seeds with fresh yeast cells of each of the two tested species increased plant height by 17.5 and 19.1%, respectively. All yield parameters were improved by the treated seeds in comparison to untreated control. Both of numbers of branches, fruit numbers of clusters and pod numbers per plant increased by 133.3, 100; 28.3, 26.7; 70.9 and 53.6% in plants initiated from seeds treated with fresh yeast cells of each of ***S. cerevisiae*** and ***T. delbrueckii***, respectively. Numbers of seeds produced and their weight per plants were also increased by 57.9 and 44.4; 88.8 and 64.9%, consecutively, in addition, seed yield per feddan increased by 58.3 and 41.7, respectively when seeds were treated with ***S. cerevisiae*** **and** ***T. delbrueckii*** **(Table 6 , Figure 3)**. Results of Mekki and Ahmed [10] showed that application of organic manure plus yeast species ***Candida tropicalis*** as one treatment resulting in an increase in yield and yield attributes of soybean plants. In this study, it was noteworthy the early maturation of the treated soybean plants in comparison to untreated one (control) which was estimated by 11 and 8 days for the treated plants with ***S. cerevisiae*** and ***T. delbrueckii,*** respectively. This early maturation may be due to capability of the tested yeast species to dissolve tricalcium phosphate. Of interest there were formation of four-seed-containing pods on the plants that the initiated seeds received treatment with yeast cells of each of ***S. cerevisiae*** and ***T. delbrueckii***. Percentage of four-seed-containing pods were estimated by 6.2 and 4.9% of total pods/plant, respectively **(plate 3)**. Whilst, the control plants could not form four-seeds containing pods.

Regarding soybean seed quality, treatment of seed with fresh yeast cells of ***S. cerevisiae*** had positively more effect than treatment of ***T. delbrueckii*** on the protein content of the produced seeds. Total protein as well as soluble and insoluble protein of seeds treated with ***S. cerevisiae*** increased by 16.47, 22.9 and 15.2 %, respectively. ***T. delbrueckii*** could increase total protein, soluble as well as insoluble protein by 4.04, 16.2 and 1.6 %, consecutively **(Table 7)**. Slightly increases in oil content of the produced seeds for the treated seeds were recorded compared with untreated control. ***T. delbrueckii*** could surpass ***S. cerevisiae*** in this regard **(Table 7). Morsy** *et al.* [12] reported that additions of ***S. cerevisiae*** at concentration 10^8 cfu/ml to the soil amended with 3% humic acid increased both of protein and oil percentage of seed yield. Effect of coating of soybean seeds with fresh yeast cells of each of the two tested yeast species on tannic acid content of soybean seeds was investigated and the obtained results showed that there was a decrease in tannic acid content of the coated seeds in comparison untreated control. This decrease was represented by 8.2 and 3.9% in case of seeds coated yeast of ***S. cerevisiae*** and ***T. delbrueckii, respectively***. **(Table 7).** Previously, critical reviews [57-59] reported that tannins (commoly referred to as tannic acid) are water soluble polyphenols that are present in many plant foods. They have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy and protein digestibility in experimental animals. Therefore, foods rich in tannins are considered to be of low nutritional values. However, recent findings indicate that the major effect of tannins was not due to their inhibition on food consumption or digestion but rather the decrease of efficiency in converting the absorbed nutrients to new body substances. Our obtained results not only offered solvability for the above mentioned limitations of use soybean as animal feed but also promising possibility of using soybean in feed with high nutritional values to improve animal productivity.

Table 4: Effect of seed coating with yeast cells of the tested yeast species on root and shoot length of seedlings, fresh and dry weights, surrounding of leaf and photosynthetic pigments of seedlings at ambient temperature after 30 days from seed germination (field experiment).

Treatment	length(cm)		fresh weight (mg/plant)		dry weight (mg/plant)		surrounding of leaf (cm)	photosynthetic pigments (mg/gm dry weight)		
	root	shoot	root	shoot	Root	shoot		Chl.a	Chl.b	Carot
untreated seeds	11.0*	26.1	290	2930	50.0	236	13.0	7.5	3.3	1.7
seeds treated with S. *cerevisiae*	15.0	32.8	550	4453	93.0	470	25.0	9.8	6.2	2.6
Effectiveness %	36.4	25.7	89.7	52.0	86.0	99.2	92.3	30.7	87.9	52.9
seeds treated with *T. delbrueckii*	16.3	35.0	440	3890	78.0	390	23.0	9.4	5.2	2.9
Effectiveness %	48.2	34.1	51.7	32.8	56.0	65.3	76.9	25.3	57.6	70.6
L.S.D at (0.05)	3.82	5.16	74.6	1140.2	22.5	53.9	5.88	1.03	0.35	0.51

S. = ***Saccharomyces*** **T. =** ***Torulaspora*** **K. =** ***Kluyveromyces*** **Chl . = Chlorophyll Carot. = Carotenoids**

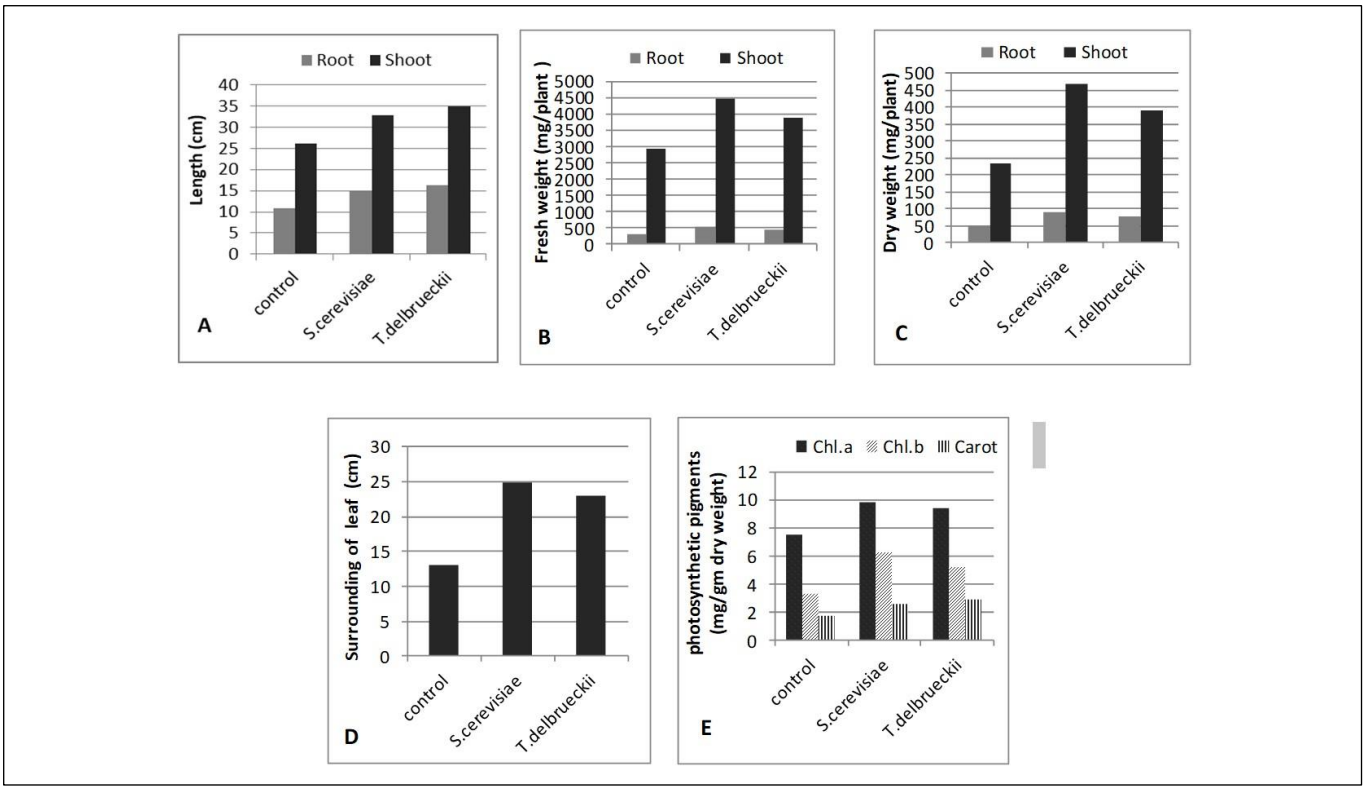


Figure 2: Effect of seed coating with the tested yeast cells on root and shoot length (A), their fresh weights (B), their dry weights (C), surrounding of leaf (D) and its photosynthetic pigments of seedlings (E)at ambient temperature after 30 days from seed germination (field experiment).

S. = *Saccharomyces* T. = *Torulaspora* Chl . = Chlorophyll Carot. = Carotenoids

Table 5: Effects of seed coating with yeast cells of the tested yeast species on number of nodules and its dry weight after 50 days from seed germination (field experiment).

Treatment	Number of nodules/ plant root	Dry weight of nodules (gm)/plant root
untreated seeds	30.3*	0.307
seeds treated with *S. cerevisiae*	50.8	0.461
Effectiveness %	67.7	50.2
seeds treated with *T. delbrueckii*	43.6	0.396
Effectiveness %	43.9	29.0
L.S.D at (0.05)	0.63	0.06

*** = average of 6 plant roots T. = *Torulaspora* S. = *Saccharomyce***

A **B** **C**

Plate 2: Flowering of untreated soybean plants (A) compared to plants treated with fresh cells of the tested yeast species *Torulaspora delbrueckii* (B) and *Saccharomyces cerevisiae* (C) after 44 days from seed germination (field experiment), (Red arrows refer to the flowers).

Table 6: Effect of seed coating with yeast cells of the tested yeast species on soybean plant growth and its productivity at the time of harvest (field experiment).

Treatment	plant height (cm)	number of branches /plant	number of fruit clusters /plant	number of pods /plant	number of seeds /plant	weight (gm) of seeds /plant	100 seeds weight (gm)	seed yield(Kg /feddan)
untreated seeds	70.3*	3.3	6.0	26.5	70.1	13.4	19.0	1400
seeds treated with *S. cerevisiae*	82.6	7.7	7.7	45.3	110.7	25.3	22.9	2216.7
Effectiveness %	17.5	133.3	28.3	70.9	57.9	88.8	20.5	58.3
seeds treated with *T. delbrueckii*	83.7	6.6	7.6	40.7	101.2	22.1	21.5	1983.3
Effectiveness %	19.1	100	26.7	53.6	44.4	64.9	13.2	41.7
L.S.D at (0.05)	10.77	0.6	0.89	1.98	4.98	1.47	1.32	239.5

*** = average of 6 plants** **S. = *Saccharomyces*** **T. *Torulaspora***

A **B**

Plate 3: Formation of four-seeds-containing pods. Zoom on four-seeds pods (A); pods on plant (B). (Red arrows refer to pods).

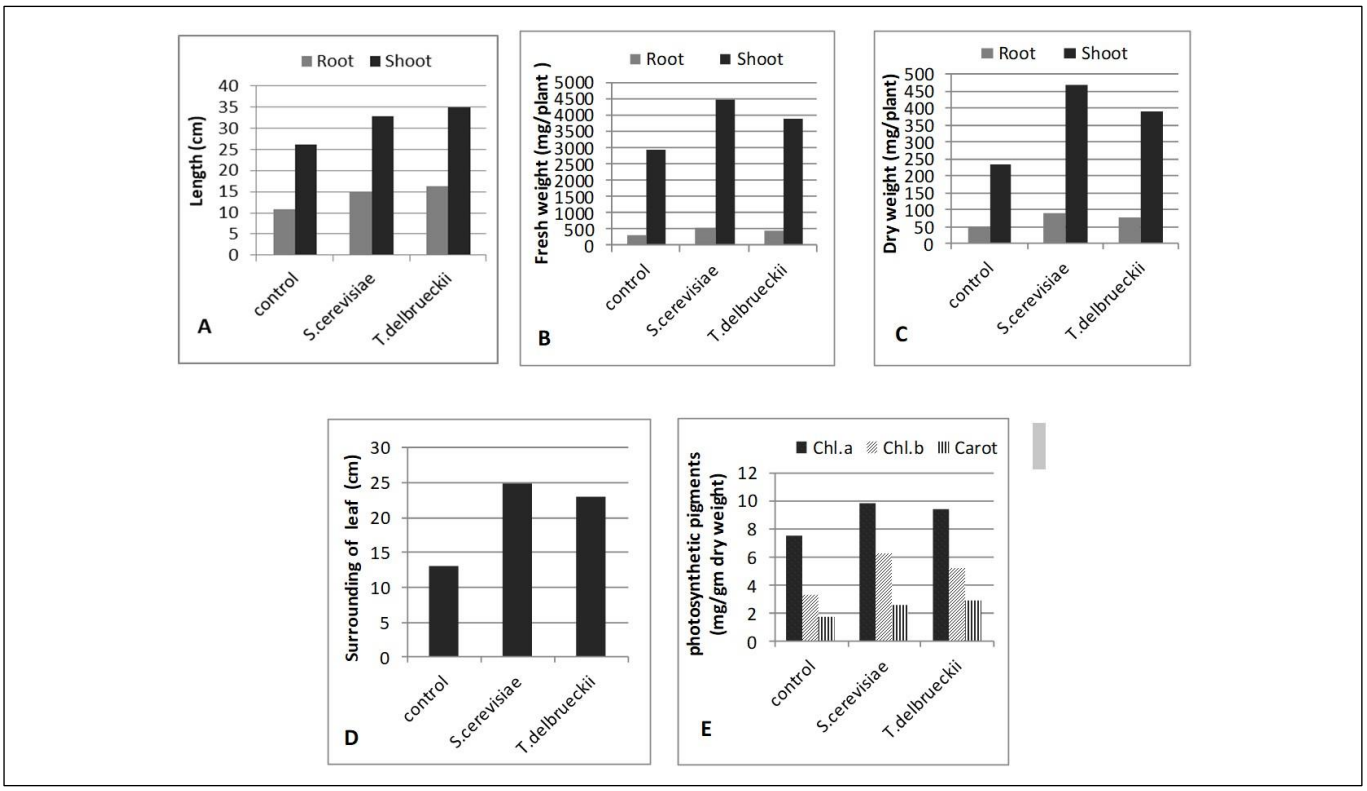


Figure 3: Effect of seed coating with the tested yeast cells on plant height (A) , their number of branches and number of fruit clusters (B), number of pods and number of seeds (C), weight of seeds/plant and 100 seeds weight (D) and seed yield/feddan at time of harvest (E) (field experiment).
T. = ***Torulaspora*** **S.** = ***Saccharomyce***

Table 7: Percentage of total, soluble, insoluble proteins and tannic acid contents as well as oil contents of the treated soybean seeds using the tested yeast species compared to untreated seeds.

Treatment	oil %	total protein (mg/gm)	soluble protein (mg/gm)	insoluble protein (mg/gm)	tannic acid (mg/gm)
untreated seeds	16	468*	77.92	390.08	2.55
seeds treated with *S. cerevisiae*	16.67	545.1	95.76	449.34	2.34
Effectiveness %	-	+16.47	+22.9	+15.2	- 8.2
seeds treated with *T. delbrueckii*	16.97	486.9	90.56	396.34	2.45
Effectiveness %	-	+ 4.04	+16.2	+1.6	-3.9

S. = ***Saccharomyces*** **T.** = ***Torulaspora*** ***** = **average of 3 seed samples**

3.6. Determination of indole-3-acetic acid production by the two tested yeast species in a chemically defined medium:

Indole-3- acetic acid (IAA) can be produced by different types of microorganisms, such as bacteria, fungi, yeasts and algae. Recently, yeasts have been received a considerable attention as IAA-producers. Several yeast taxa were investigated for this purpose such as ***Pichia spartinae*** [60] ***Candida valida***, ***Rhodotorula glutinis*** and ***Trichosporon asahii*** [2], ***Cyberlindnera (Williopsis) saturnus*** [4], ***Rhodotorula graminis*** and ***R. mucilaginosa*** [14], ***Candida tropicalis*** [22], ***Cryptococcus*** sp. [61], ***Candida maltosa*** [62]. ***Saccharomyces cerevisiae*** [7], ***Rhodosporidium fluviale*** [63] and ***Rhodosporidium paludigenum*** [16], therefore, yeasts are one of the most interesting IAA-producers. Obtained results of our investigation for *in vitro* indole-3-acetic acid production in synthetic medium supplemented with 0.1% L-tryptophan at 28ºC using ***Sacchoromyces cerevisiae*** and ***Torulaspora delbrueckii*** revealed that both two tested yeast species could produce indole-3-acetic acid and their high production values were estimated by 85.4 and 115.4 µg/ml at the third day of incubation, respectively **(Table 8, Figiure 4)**. Of interest was the capability of the two tested yeast species to produce *in vitro* indole-3-acetic acid in medium without L-tryptophan. Maximum production of it was determined by 57.4 and 50.6 µg/ml after 5 days incubation period in case of ***S. cerevisiae*** and ***T. delbrueckii***, respectively **(Table 8, Figiure 4)**. As previously presented results (**Tables 2 and 6)** of the promoting effects of ***S. cerevisiae*** and ***T. delbrueckii*** on soybean plant growth and yield traits , it might be mainly related to their abilities to produce considerable amounts of indole-3-acetic acid. Indole-3-acetic acid has a number of important roles in plants, such as the stimulation of cell division, cell elongation, cell differentiations, light and gravitational responses, regulating leaf fall and fruit ripening [64-65] and offers the increased protection of plants in presence of external stress [66]. Our results obviously ensure that ***S. cerevisiae*** and ***T. delbrueckii*** can promote soybean plant growth and its yield traits as well as seed quality and support ongoing prospecting of yeasts as promising successful fertilizer for sustainable agriculture which might be according to their abilities for the high production of Indole-3-acetic acid.

3.7. In vitro solubilization of insoluble inorganic phosphate by the two tested yeast species:

Phosphorus represents one of the most important major elements for plant nutrition and development. It plays several and important roles in the plant life. These roles include accumulation and release of energy during cellular metabolism and enter in many organic compound. It is added to soils in form of mineral or inorganic manner. In case of its entrance as inorganic phosphorus form it is rapidly fixed as tricalcium phosphate and become unavailable for plant nutrition, particularly in alkaline soils with high percentage of calcium carbonate. Fortunately, many soil microorganisms including bacteria, fungi and yeasts have the ability to transform insoluble forms to soluble forms ready for plant nutrition [67]. Results of our investigation for capability of ***S. cerevisiae*** and ***T. delbrueckii*** to dissolve tricalcium phosphate in a chemicallydefinedmedium showed that the two tested yeast species possess abilities to dissolve tricalcium phosphate with dropping pH of the culture broth from 6.5 to 5.3 and 5.2, at 28ºC after 10 days incubation, respectively, **(Table 9, Figure 5)**. Amounts of dissolved phosphate by ***S. cerevisiae*** and ***T. delbrueckii*** were estimated by 400 and 486 µg/ml after incubation for 10 days at 28ºC. Microorganisms are known to dissolve insoluble phosphate through the production of organic acids [67] and these acid lower pH of the medium and bring about the dissolution of bound forms of phosphate. Sharma *et al*. [68] reported that an adequate supply of phosphorus during early phases of plant development is important for laying down the primordial of plant reproductive parts. It plays a significant role in increasing root ramification and strength there by importing vitality and disease resistance to plant. Also it helps in seed formation and early maturation of crop like cereals and legumes [59]. Narsian *et al*. [21] reported that two strains of ***Saccharomyces sp***. could solubilize tricalcum phosphate with dropping pH of broth medium to 4.6 and 5.7 and maximum P solubilization 21.2 and 45.44 mg% P_2O_5. Fu *et al*. [69] suggested that ***S. cerevisiae*** JYC 400 and ***Torulaspora*** sp. JYC 486 exhibited *in vitro* tricalcium phosphate solubilizing activity. According to all previous mentioned reports our results referred to the potentiality of the two tested yeast species in dissolving tricalcium phosphate exhibiting their ability to be used as soil inoculants for improving soil fertility and plant growth.

3.8. *In vitro* production of polyamine (s) by the two tested yeast species in a chemical defined medium:

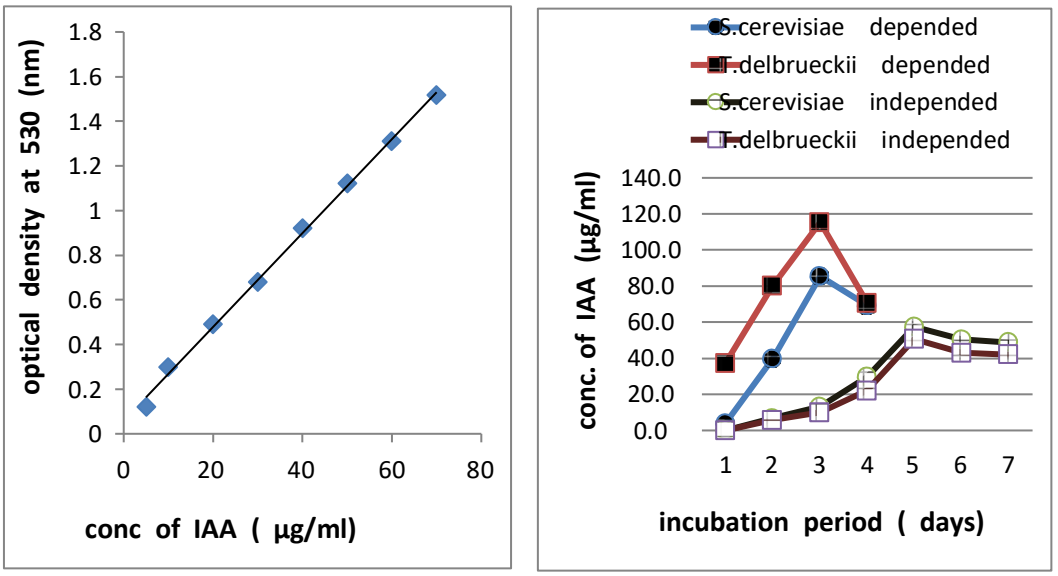
A polyamines is an organic compound containing two or more primary amino group and is involved in several steps of plant development [70], Polyamines also play an important role in plant tolerance against biotic and abiotic stresses [71]. Our investigation of potentialities of ***S. cerevisiae*** and ***T. delbrueckii*** to produce *in vitro* polyamine(s) during growth in a chemically defined medium revealed that both species possessed the capability to produce polyamine(s) with different concentrations which were determined through the obvious difference in the pink color intensity at 530 nm optical density. Values of color intensities test were estimated by 0.81 and 1.19 for ***S. cerevisiae*** and ***T. delbrueckii***, respectively **(Table 10)**. ***T. delbrueckii*** surpassed ***S. cerevisiae*** in its capability of polyamine production. Ability of both yeast species to produce polyamine(s) may support our results of increase of all soybean plant growth parameters and yield traits for the treated seeds compared to untreated control, since the exogenous application of polyamines are known to impact on root growth, whereas, Sharma and Ali [72] pointed out that polyamines act as modulators of soybean productivity. In addition, they reported that polyamines increased numbers of pods per plant, 100 seed weight, seed yield and oil yield. Therefore, polyamines can be used to increase soybean productivity. Tang and Newton [73] showed that polyamines promote root elongation and growth by increasing root cell division in regenerated Virginia pine plantlets. Cloete *et al*. [11] showed that ***Cryptococcus laurentii*** could increase root growth of sclerophyllous medicinal shrub ***Agathosma betulina*** by 51%. Further studies are required to determine quality as well as quantity of polyamine(s) produced by the two tested yeast species.

In conclusion, ***S. cerevisice*** and ***T. delbrueckii*** could promote soybean plant growth, its productivity and enhance seed quality and quantity through different mechanisms such as indole-3-acetic acid production, phosphorus solubilization and polyamine production. Further studies should be planned in the future to investigate the other non-tested mechanisms of action before application of these environmentally, friendly promising yeasts as biofertilizers for sustainable agriculture.

Table 8: *In vitro* indole-3-acetic acid production by the two tested yeast species (***S. cerevisiae*** and ***T. delbrueckii***) in a chemical defined medium.

Yeasts species/media	IAA (µg/ml) after incubation time (days)						
	1d	2d	3d	4d	5d	6d	7d
S. cerevisiae **tryptophan-dependant**	4	39.7	85.4	69.2	-	-	-
S. cerevisiae **tryptophan-independant**	0	6.8	13.1	29.7	57.4	50.4	48.8
T. delbrueckii **tryptophan-dependant**	37.3	80.2	115.4	70.7	-	-	-
T. delbrueckii **tryptophan-independant**	0	5.7	10.0	22.1	50.6	43.0	42.1

IAA = indole -3- acetic acid **S. = *Saccharomyces*** **T. = *Torulaspora***



A **B**

Figure 4: Standard curve of indole-3-acetic acid (A) and *In vitro* indole-3-acetic acid production by the two tested yeast species *S. cerevisiae* and *T. delbrueckii* (B) in a chemical defined medium with or without 0.1% L- tryptophan.

T. = *Torulaspora* **S. = *Saccharomyces***

Table 9: *In vitro* solubilization of tricalcium phosphate by the two tested yeast species (***S. cerevisiae*** and ***T. delbrueckii*****)** after 10 days incubation at 28ºC.

tested yeasts species	final pH of cultured medium	phosphate solublization µg/ml
S. cerevisiae	5.3	400
T. delbrueckii	5.2	486

S. = *Saccharomyces* **T. = *Torulaspora***

Table 10 : In vitro production of polyamines by the two tested yeast species (***S. cerevisiae*** and ***T. delbrueckii*****)** in a chemically defined medium

tested yeast species	intensity of pink color at optical density 530 nm
S. cerevisiae	0.81
T. delbrueckii	1.19

S. = Saccharomyces ***T. = Torulaspora***

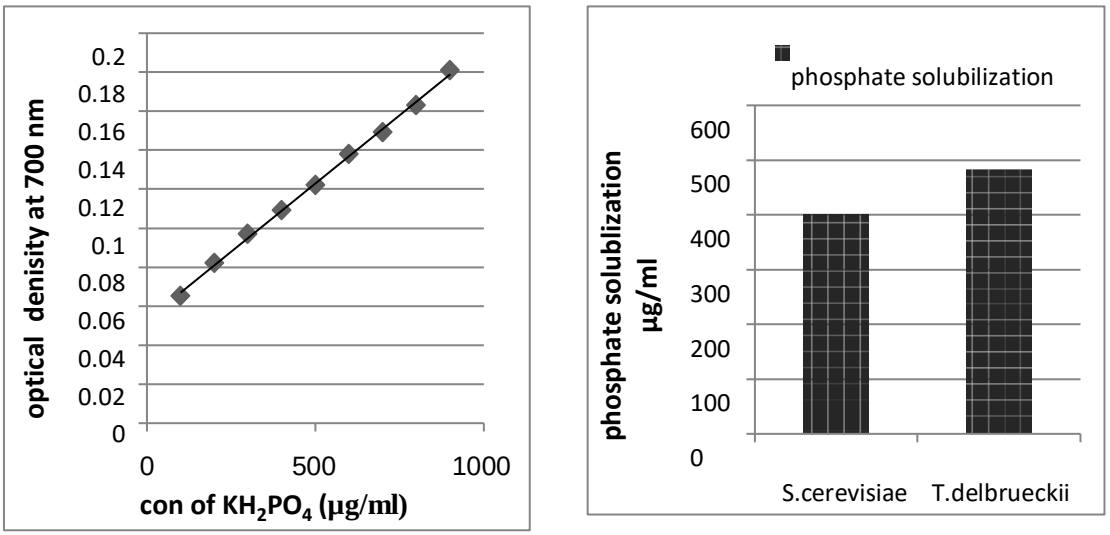


Figure 5: Standard curve for soluble phosphate (A) and *In vitro* solubilization of tricalcium phosphate by the two tested yeast species *S. cerevisiae* and *T. delbrueckii* (B) after 10 days incubation at 28ºC.

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