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EFFECT OF FOLIAR SPRAYING OF SOME YEAST MUTANTS ON VALENCIA ORANGE TREES (*Citrus sinensis*)

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ABSTRACT

Saccharomyces cerevisiae mutants selected after EMS treatments were evaluated for their efficiency in improving growth, nutritional status of trees, preharvest fruit drop percentage, yield and quality of Valencia orange fruits. Mutants were selected according to their proline accumulation and osmotolerance in the presence of a solution of high osmolarity (1 M NaCl). Two mutants, which were more osmotolerant and had approximately 10 times more proline than the original strain, were selected for foliar spraying of Valencia orange trees at a concentration of 1 ml (4 X 10^7 cells)/L. The experimental trials were conducted three times during 2001/2002 and 2002/2003 in a private farm in Quwesna, El-Menofeya Governorate, Egypt. Results showed a remarkable improvement in leaf area, leaves mineral content, preharvest fruit drop percentage and yield. Physical and chemical properties of fruits were also enhanced in both harvest dates. Quality of fruit especially vitamin C and amino acid contents were also increased. It could be recommended for foliar spraying by yeast mutants especially for exporting Valencia orange fruits.

INTRODUCTION

Citrus industry in Egypt, nowadays, proved to be the most important fruits grown in both the old valley and new reclaimed areas. Its plantations reached about 344789 fed., with total fruits production of 2594853 ton. (Min. of Agric. Stati., 2001). Citrus fruit considered as the best Popular fruit, because of its nice taste, excellent flavour, high content of vitamins, moreover, nutritional and medical constituents. Many food industries such as juice, jam, pectin and oil with its pharmaceutical effect. Furthermore, citrus fruits exported for many countries, specially that produced under organic and biofertilization.

Biofertilization has become in the last few decades a positive alternate to chemical fertilizers. Biofertilizers are very safe for human, animal nutrition and in harmony with environment. The bio-fertilization cultivation method used different micro-organisms including bacteria, fungi and yeast. Foliar spray with active dry yeast on fruit plants has recently received apparent interest. The various positive effects of applying active dry yeast were attributed to its contents of different nutrients, higher percentage of proteins, large amount of vitamin B and natural plant growth hormones, namely, cytokinins. In addition, application of active dry yeast is very effective in releasing CO_2 which improves net photosynthesis (Larson *et al.*, 1962; Ferguson *et al.*, 1987 and Idso *et al.*, 1995).

The possibility of using yeast *S. cerevisiae* for improving growth and productivity of fruit crops was mentioned by Subba Rao (1984), Nijjar (1985), Hegab *et al.* (1997), Abdalla *et al.* (1998) and Nomier (2000).

An important issue for basic research and applied biotechnology is the mechanism of cellular responses when cells are exposed to adverse environmental stresses, including freezing, desiccation and high osmolarity (Morita *et al.*, 2003). The best-characterized biochemical response of bacterial and plant cells to osmotic stress is the production and accumulation of osmoprotectants such as trehalose, glycerol, betaine and proline. (McCue and Hanson, 1990). Among such molecules we focused on proline as a cryoprotectant because it resulted in increased tolerance to freezing in yeast cells. In addition, proline is known to play an important role as an osmoprotectant in plants subjected to hyperosmotic stresses such as drought and soil salinity (Hong *et al.*, 2000).

The aim of this investigation tended to study the effect of foliar sprays by two proline overproducing *S. cerevisiae* mutants on citrus growth, fruit production as well as fruit content of amino acids.

MATERIALS AND METHODS

This investigation was performed during two successive seasons, 2001/2002 and 2002/2003 on eight years old Valencia orange tress (*Citrus sinensis*), buded on sour orange (*Citrus aurantium*, L.) root stock which spaced at 5 X 5 meters in a loamy soil (Table 1) of a private farm at Quwesna El-Menoufya Governorate.

Table (1) : Physical and chemical properties of the used soil.

1. The physical analysis :	
Sand %	23.47
Silt %	19.33
Clay %	57.20
Texture grade	Loam
2. The chemical analysis :	
pH (1:2.5 exract)	9.04
EC, mmhos/cm (1:5)	0.22
Organic matter %	1.61
$CaCO_3$ %	1.07
3. Available macro elements (meq/100 g) :	
P	2.21
K	22.00
Mg	329.39
Ca	240.80
Na	154.27
4. Available microelements (ppm) :	
Mn	2.23
Zn	3.50
Fe	6.80
Cu	2.67

Nine uniformed trees were chosen randomly as replicates in a complete randomized block design and grouped under three treatments including control. Each treatment contained three replicates (3 trees), each tree as a replicate. The chosen trees were subjected to the normal horticultural practices used in this farm.

Yeast strain :

Culture of yeast *Saccharomyces cerevisiae* was kindly provided by the Egyptian company for yeast and starch, Alexandria.

Media :

The minimal medium (MM) used was yeast nitrogen base without ammonium sulfate. For isolation of proline-nonutilizing mutants, MM was supplemented with 0.5 % galactose as the sole source of carbon and 0.1 % of glutamate or proline as a source of nitrogen.

SD medium, containing 2 % glucose, 0.67 % Bacto-yeast nitrogen base without amino acids, was used for osmotolerance test.

YPD medium, containing 2 % glucose, 2 % peptone and 1% yeast extract.

Ethyl Methan Sulfonate (EMS) mutagenicity :

EMS (1.06 M) was obtained from Sigma and treatment was performed according to standard protocols (Rose *et al.*, 1990 and Winston, 1992). A single colony was picked and grown in 5 ml liquid YPD medium for 18 h at 30°C with continual rotation at 200 rpm to allow sufficient time for culture to reach stationary phases). One ml of cell suspension (4×10^7 cells) was treated with 25, 50, 100 or 200 µl/ml cell suspension for 6 hours at 30°C with agitation. Appropriate dilutions of the mutagenized cultures were plated on MM containing galactose and glutamate and incubated for four days at 30°C. Each grown colony was picked up and transplanted on slants for further genetic analysis.

Isolation of proline-nonutilizing mutants:

Mutagenized cells of *S. cerevisiae* were plated on agar plates containing galactose and glutamate and were incubated at 30°C for 3-5 days. Colonies were replica plated to galactose-proline and galactose glutamate plates and incubated at 30°C for 2 days. Colonies which failed to grow on the galactose-proline plates but grew on the galactose-glutamate medium were selected.

Galactose was used as the carbon source to select against respiratory–deficient yeast mutants which are proline nonutilizer due to their lack of mitochondrial function.

Osmotolerance of yeast mutants and amino acid contents :

For osmotolerance test, mutants were grown in 5 ml of SD with 1 M NaCl and incubated at 30°C for 48 h. Cell growth was measured by optical density at 570 nm (OD_{570}). The intracellular quantities of amino acids were determined according to Morita *et al.* (2003) with an amino acid analyzer (L3000, Eppdrof Germany), and expressed as percentages of the dry weight.

Foliar spray treatments :

The foliar spray treatments were as follows :

1. Control (untreated).
2. Trees sprayed with mutant A
3. Trees sprayed with mutant B

Valencia orange tress were sprayed three times in each season. The first spray was done when fruit diameter reached to 1.2 to 1.8 mm, i.e., at 2nd week of May, while the second spray was at the 4th week of June, and the third one was at the 1st week of August. All spray solutions contained one ml of yeast suspension (4 X 10^7 cells) per liter and 0.1 % of triton B as a wetting agent. The trees were sprayed till run off (5 L/tree).

Leaf area (cm²) was measured on mid-September by planimeter, on 30 full mature leaves/trees (from the 4th to 5th leaves from the shoot base) as noted by (Nautiyal *et al.*, 1990). Samples of 30 mature leaves from each shoot were selected randomly from each replicate, dried and prepared to determine their elemental contents of N, P, K and Ca (according to Evenhuis and Dewaard, 1980).

Preharvest drop percentage was calculated by counting the number of dropped fruits from mid February till the commercial harvesting date under the experimental conditions, i.e. during two times, the first was the last week of February, while the second one at 1st week of April.

The productivity : [Yield/tree (kg)] was estimated at harvest time (1st week of April) as follows : Total number of fruits per tree at harvesting time X average weight of fruit in g. Meanwhile, feddan productivity (ton) was calculated according to the following equation =

$$\frac{\text{the average yield/tree(kg)X160 tree}}{1000}$$

Sample consisting of thirty fruits was randomly taken at harvest time from each replicate for determining the physical and chemical properties of fruits i.e., average of fruit weight (g), average of fruit size (cm^3), fruit dimensions, rind thickness (mm), juice weight (gm), juice %, total soluble solids and total acidity expressed as (gm) citric acid per (100 gm) Pulp], were determined as mentioned in A.O.A.C. (1980).

Vitamin C : (Ascorbic acid "mg/100 ml juice") was determined according to the modified method of Omaye *et al.* (1979). Plant material was homogenized with 5 % metaphosphoric acid and the color reagent was 2,6-Dichlorophenolindophenol. The absorption of the sample at 525 nm relative to the blank was measured. Standard curve was constructed using L-ascorbic acid in the range of 0 to 4 mm.

Analysis of fruit's amino acids :

Amino acids were analysed according to the method of Moore and Shtaien, (1954) using High Performance Amino Acid Analyzer L3000 (Eppdrof-Germany) with the following condition : flow rate, 0.2 ml/min, pressure of buffer, 0 to 50 bar, pressure of reagent, 0-150 and reaction temperature was 123°C.

Data were statistically analyzed in a complete randomize blank design, using Duncun's method to differentiate means at 0.05 according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Induction and isolation of proline nonutilizing mutants :

Four ethyl methane sulfonate concentrations; 25, 50, 100 and 200 µl/ml were used to treat *S. cerevisiae* for 6 hours in addition to the control treatment. Results showed that mutagenized cells grew at a slower rate for all treatments of EMS. Fig. (1) illustrated the effect of various concentrations of EMS on survival rate. It is clear that survival percentage decreased sharply by increasing EMS concentrations. It was 13.62, 4.80 and 0.75 % after treatment with EMS at concentrations of 25, 50 and 100 µl/ml, respectively. Treatment with 200 µl of EMS effectively killed the cells. The observed fitness reduction may be due to toxic effects of EMS rather than DNA damage and mutation (Mable and Otto, 2001).

Proline-nonutilizing mutants were isolated as described under materials and methods. Brandriss and Magasanik (1979) isolated proline-nonutilizing mutants of *S. cerevisiae* and proved that mutants in *put* 1 are deficient in proline oxidase and those in *put* 2 lack P5C dehydrogenase. However, in *S. cerevisiae* the PRO gene is known to be constitutively expressed and both γ-GK and γ-GPR are rate-limiting enzymes in yeast (Brandriss and Flavey, 1992).

Morita *et al.* (2003) observed two basic types of mutants in *S. cerevisiae*. One type involves a dominant mutation directly linked to proline accumulation, which is due to enhancement of preexisting enzymes activities or to upregulation of the gene expression involved in proline biosynthesis. The other type of mutants involves a recessive mutation that does not increase the proline content compared to that of the parent, suggesting that a mutation may occur in the proline permease (Lasko and Brandriss, 1981) or in membrane composition.

Intracellular amino acid contents of mutants :

Microorganisms that overproduce various amino acids have been constructed by conventional mutation techniques (Matsutani *et al.*, 1990) or by isolating mutants resistant to analogues of corresponding amino acid (Takagi *et al.*, 1997).

The intracellular quantities of amino acids in mutants and their parental strain were determined by an amino acid analyser. In most cases, the variation was in proline, glutamate and/or arginine, which all or some of them were higher in the mutant cells than in the parent (Table 2). It is clear that about 65 % of the mutants were found to accumulate a higher amount of proline in the cells than their parental strain. The best mutant (No. 11), had approximately 10 times more praline content. In general, mutants can be divided into the following three groups : those whose proline content only increased, those whose proline as well as glutamate and arginine contents increased, and those whose two amino acids contents were only increased compared to those of the in parental strain.

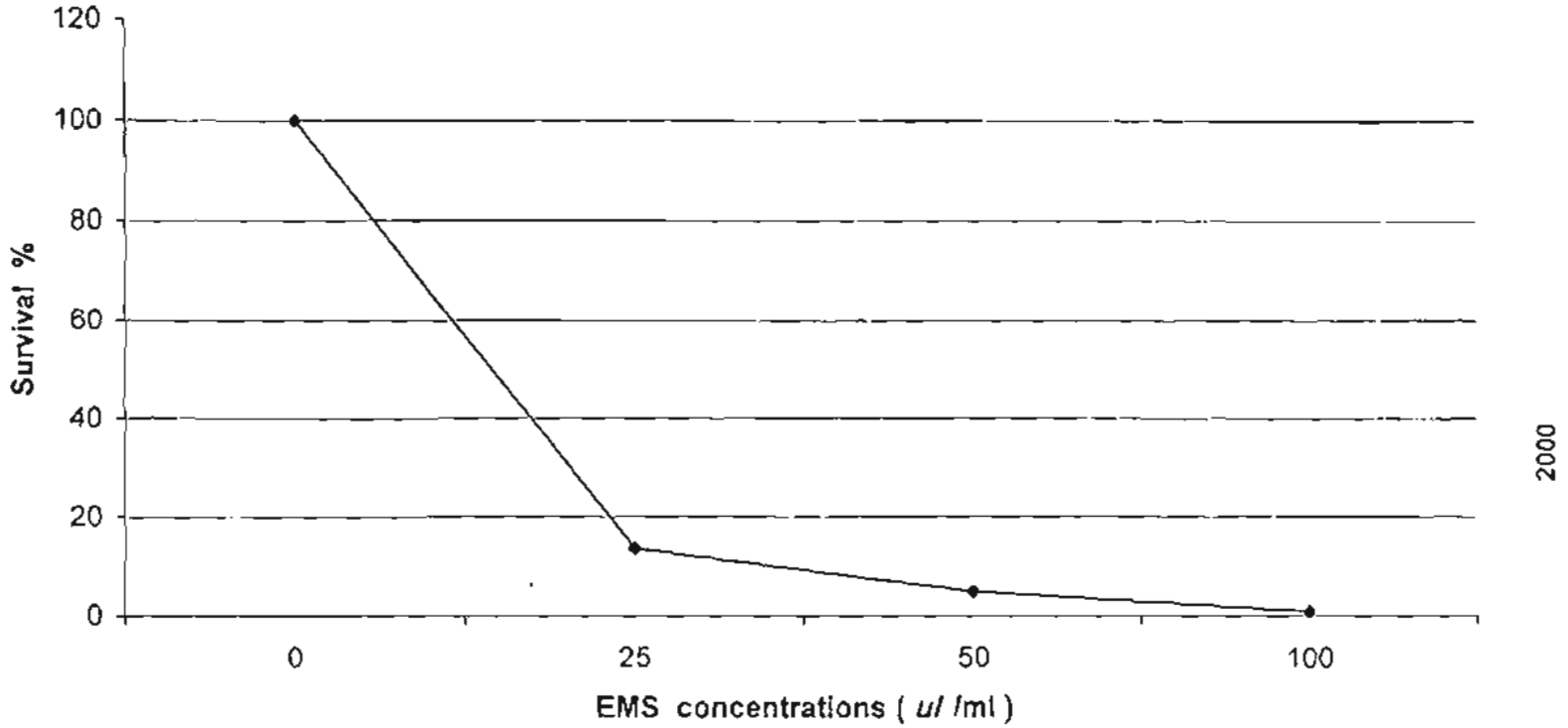

Fig.1 Survival percentages of *S.cerevisiae* after treatment with different concentrations of EMS for six hours

In some mutants, glutamate and arginine which related to proline metabolism as well as proline were accumulated. This may be due to the mutation upstream of the proline metabolic pathway (Takagi *et al.*, 1997).

Osmotolerance of mutants :

In many bacteria and plants, the intracellular proline content increases in response to environmental osmotic stress (Buhl and Stewart, 1983 and Kawahara *et al.*, 1989). This intracellular accumulation of natural organic solutes occurs in order to prevent membrane damage during the removal of water (Takagi *et al.*, 2000).

Table (2) : Intracellular amino acid contents of EMS mutants.

Strain	Treatment	Amino acid (% of dry weight)		
		Glu.	Arg.	Pro.
Parental strain	-	3.52	2.25	0.64
Mutant No.1	25 µl EMS/ ml	3.75	2.23	0.83
" 2		1.25	1.27	5.01
" 3		2.39	1.87	3.72
" 4		0.92	1.44	4.65
" 5		0.62	1.02	0.67
" 6		3.25	6.20	4.38
Mutant No.7	50 µl/EMS ml	5.88	4.80	5.88
" 8		1.98	2.93	0.69
" 9		1.25	1.68	5.44
" 10		8.0	5.88	2.18
" 11		3.01	4.08	6.57

In this study, the growth of various mutants that accumulate a higher amount of proline during high osmotic stress imposed by 1 M NaCl was examined (Fig. 2). Mutant No. 11, which had a significantly higher initial proline level, was clearly more osmotolerant than other strains in the presence of a solution of a high osmolarity. Accordingly, it was observed that most mutants were also able to grow under osmotic stress. These results indicate that intracellular proline would function as osmoprotectant effect. Proline-overproducing mutants of *E. coli* (Dandekar and Uratsu, 1988); *S. typhimurium* (Csonka, 1981). *S. marcescens* (Suglura and Kisumi, 1985) and *S. cerevisiae* (Takagi *et al.*, 1997) clearly show enhanced osmotolerance.

On the bases of the above results mutants No. 7 and 11, which were designated A and B, respectively, were chosen to foliar spray of Valencia orange trees.

Effect of yeast mutants foliar applications on leaf area and leaf mineral contents :

It is clear from data in Table (3) that spraying Valencia orange trees with two yeast mutants (A & B) were very effective in increasing the leaf area. The highest significant results were noticed by foliar application of mutants A & B which showed (9.73, 8.65) and (10.61, 9.31 cm^2) in both seasons, respectively. The lowest significant leaf area was obtained from control (8.15 and 8.70 cm^2) consecutively during 2001/2002 and 2002/2003 seasons.

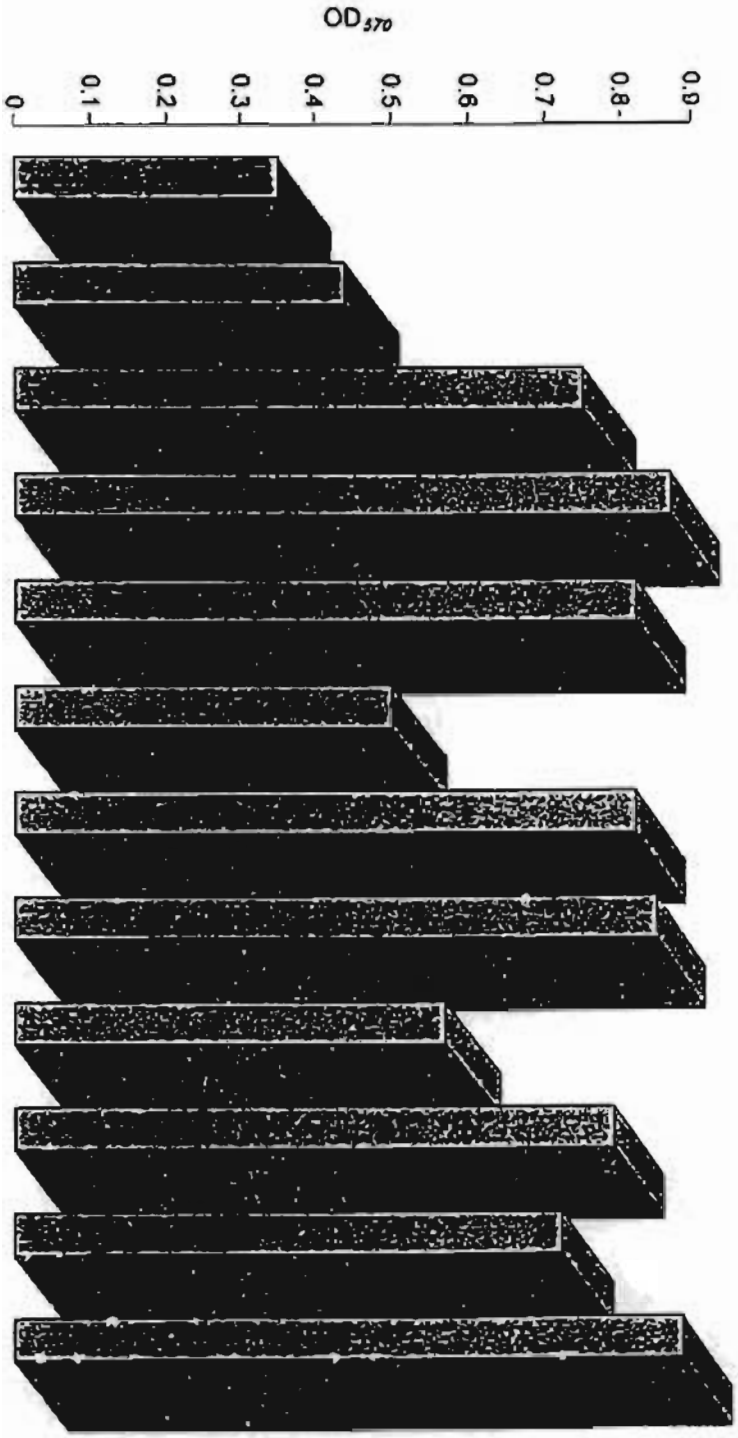

FIG.2 Growth of EMS mutants and their parental strain under high osmotic conditions

Table (3) : Effect of spraying the two mutants of yeast on the leaf area (cm^2) and leaf mineral contents of Valencia orange trees during 2001/2002 and 2002/2003 seasons.

Characters	Leaf area (cm^2)		N %		P %		K %		Ca %	
Treatments	2001/2002	2002/2003	2001/2002	2002/2003	2001/2002	2002/2003	2001/2002	2002/2003	2001/2002	2002/2003
Control	8.15 b*	8.70 b	3.02 c	3.83 c	0.10 a	0.11 a	0.65 c	0.65 b	1.40 c	1.51 b
Trees sprayed with mutant A	9.73 a	10.61 a	3.18 a	5.03 a	0.11 a	0.12 a	0.75 a	0.75 a	1.45 b	1.50 b
Trees sprayed with mutant B	8.65 b	9.31 ab	3.16 b	4.14 b	0.12 a	0.11 a	0.70 b	0.75 a	1.50 a	1.55 a

*Values followed by the same latter in each column are not significantly different at 5 % level according to Duncan's Multiple range test.

Regarding the effect of such factors under this study, the N percentage in leaves indicated higher significant levels with mutant A (3.18 & 5.03 %) followed by mutant B (3.16 & 4.14%) compared to the control (3.02 & 3.83 %) throughout the two seasons, respectively.

Phosphorus level in leaves revealed insignificant differences between treatments in both seasons.

K level showed significant differences between treatments in both seasons. Trees sprayed with mutant A gave the highest K level (0.75 %), while trees sprayed with mutant B was the next (0.70 %) in the first season. However, there was no difference in the levels between the two mutants (A & B) in the second season. The control showed the lowest significant K level in both seasons.

For leaf Ca content, the highest values (1.50 & 1.55 %) were obtained from trees sprayed with mutant B in both seasons, respectively. Mutant A came the next, with no significant difference between the control and such treatment in the second season only.

Such increase in leaf area, as well as, leaf mineral contents could be considered as signs of general promotion on vegetative growth as a result of foliar application by yeast which may be attributed to its content of different nutrients, higher percentages of proteins, higher values of vitamins, especially vitamin B which plays an important role in improving growth and controlling the incidence of fungi diseases, as mentioned by Ingram (1958); Meyer & Phaff (1969) and Subba Rao (1984).

These results are also in agreement with those reported by Hegab *et al.* (1997) on Valencia orange trees, Ahmed *et al.* (1997) on Roumy Red grapevines, El-Mogy *et al.* (1998) and Nomier (2000) on Thompson seedless grapevines, stating that bio-fertilizing with active dry yeast showed remarkable improvements in all growth aspects such as leaf area and leaf mineral contents.

Effect of foliar applications with yeast mutants cell suspensions on pre-harvest drop percentage, yield and yield components :

Table (4) clearified that foliar application with two yeast mutants reduced preharvest fruit drop percentage when compared with control, but without significant differences between such treatments in both seasons.

The number of fruits/tree showed nearly similar trend as shown for pre-harvest drop (%). Data showed that trees sprayed with mutants A & B gave the highest number of fruit/tree; (195.33, 188.67) & (195.33, 192.33) as compared with the control (175.33, 187.67) during both seasons, respectively.

Considering the early picked date, trees sprayed with mutant B gave the highest significant average of fruit weight (199.10 & 203.48 gm), in both seasons, respectively. Trees sprayed with mutant A developed (164.94 & 170.76 gm), while the control were (162.25 & 164.58 gm) in both seasons, respectively with highest significant differences between them. As for the later picked date, the results revealed that both mutants (A & B) had significantly increasing in the average of fruit weight (194.36 & 191.67 gm) and (195.03 & 198.28 gm) comparing with their control (166.58 & 167.32 gm), in both in both seasons, respectively.

Data in Table (4) indicate that spraying the trees with the two mutants A & B gave a significantly higher yield/tree (kg) during both seasons. Meanwhile, the control trees gave the lowest yield/tree (kg). Feddan productivity (ton) showed a nearly similar trend as shown for the yield/tree.

These results might be due to the positive effect of spraying yeast which might be containing some natural plant growth regulators such as cytokinin and containing 4.92 % cellulose and higher Ca content which prevented the formation of the abscission zone as well as the abscission layer (Ingram, 1958, Meyer and Phaff, 1969 and Subba Rao, 1984).

Furthermore, the increase in number of fruit/tree, average of fruit weight and yield/tree by spraying yeast might be due to its content of tryptophan (Abdel-Latif, 1987), which is precursor of IAA (Wareing and Phillips, 1973; Moor, 1979).

Physical characteristics of fruits as affected by foliar application with yeast mutants :

Table (5) obviously indicate that fruit length and its diameter showed highly significant differences, after treatment with mutant B, compared with control (7.21 & 7.29 cm for length and 7.17 & 7.21 cm for diameter in the two seasons, respectively). Mutant A came the next but with no significant difference compared with the control.

Although the two mutants of yeast improved the fruit size (180.00 & 229.33 cm^3, consecutively), the increase in size was insignificant comparing with their control (174.67 cm^3), in the first season. However, in the second season, the average fruit size significantly increased by the two treatments. The largest significant increase in fruit size achieved by spraying with mutant B reaching 229.33 cm^3, followed by spraying with mutant A (recorded 184.00 cm^3), the control treatment recorded the smaller significant fruit size (171.00 cm^3).

Table (4) : Effect of spraying the two mutants of yeast on the preharvest fruit drop percentage, number of fruit/tree, average of fruit weight (gm), yield/tree (kg) and feddan productivity (ton) of Valencia orange trees during 2001/2002 and 2002/2003 seasons.

Characters / Treatments	Preharvest fruit drop (%)		No. of fruit per tree		Average of fruit weight (gm)				Yield/tree (kg)		Feddan productivity (ton)	
					2001/2002	2001/2002	2002/2003	2002/2003				
	2001/2002	2002/2003	2001/2002	2002/2003	1st harvest time	2nd harvest time	1st harvest time	2nd harvest time	2001/2002	2002/2003	2001/2002	2002/2003
Control	1.90 a*	2.25 a	175.33a	187.67a	162.25 b	166.58 b	164.19 b	167.32 b	29.19 b	31.24 b	4.67 b	5.00 a
Trees sprayed with mutant A	1.53 a	1.77 a	195.33a	188.69a	164.94 b	194.36 a	170.76 b	191.67 a	38.17 a	36.15 a	6.11 a	5.78 ab
Trees sprayed with mutant B	1.03 a	1.06 a	195.33a	192.33a	199.10 a	195.03 a	203.48 a	198.28 a	38.08 a	38.16 a	6.10 a	6.11 a

*Values followed by the same latter in each column are not significantly different at 5 % level according to Duncan's Multiple range test.

Table (5) : Effect of spraying the two mutants of yeast on some physical properties on Valencia orange trees during 2001/2002 and 2002/2003 seasons.

Characters / Treatments	Fruit length (cm)		Fruit diameter (cm)		Average of fruit size (cm²)		Average of fruit thickness (mm)		Average of juice weight (gm)				Juice (%)			
									2001/2002		2002/2003		2001/2002		2002/2003	
	2001/2002	2002/2003	2001/2002	2002/2003	2001/2002	2002/2003	2001/2002	2002/2003	1st harvest time	2nd harvest time	1st harvest time	2nd harvest time	1st harvest time	2nd harvest time	1st harvest time	2nd harvest time
Control	6.82b*	6.82b	6.60a	6.56b	174.67a	171.00c	4.15b	3.93b	92.85b	152.38a	98.43b	154.29a	57.23a	81.87a	60.77a	92.47a
Trees sprayed with mutant A	6.85b	6.86b	5.81b	6.81b	180.00a	184.00b	5.27a	5.07a	92.80b	183.13a	97.11b	169.52a	55.63a	93.88a	56.90a	88.50a
Trees sprayed with mutant B	7.21a	7.29a	7.17a	7.21a	290.67a	229.33a	5.47a	5.13a	114.23a	188.17a	133.43a	180.45a	57.55a	96.42a	55.75a	90.81a

*Values followed by the same latter in each column are not significantly different at 5 % level according to Duncan's Multiple range test.

Regarding the average of rind thickness, both mutants of yeast gave similar statistically results in this respect, except the control which showed the lowest significant rind thickness, in both seasons.

Data in Table (5) showed that spraying the two yeast mutants (A & B) significantly improved juice weight comparing with their control, in both seasons. Concerning, the early picking date, mutant B gave the highest significant increasing in juice of weight 114.23 and 133.43 gm in the two seasons, respectively while, mutant A did not indicate any significant improve when compared with the control. At the late picking time, both mutants A & B gave the highest juice weight (183.13, 169.52 gm) & (188.17, 180.45 gm) while the control gave (152.36, 154.29 gm) consecutively, in the two seasons, respectively. In addition, similar trend was obtained concerning juice percentage.

The previous results as affected by two mutants of yeast, are in harmony with those found by Ahmed *et al.* (1997) on Red Roomy grapevines who demonstrated that berries dimensions were significantly improved by spraying with active dry yeast. Also, the results obtained on the effect of spraying with yeast one some fruit physical characteristics were in agreement with the results obtained by Hegab *et al.* (1997) and Abdalla *et al.* (1998) who worked on Valencia orange fruits. The same trend of results was obtained by Mansour (1998) on Anna apples.

Fruit chemical characteristics :

Data presented in Table (6) showed that total soluble solids percentage revealed insignificant differences between treatments in both harvesting dates, during the two seasons.

Generally, as for acidity, all conducted treatments gave high levels in the early harvest date. Meanwhile, it revealed low levels in the late date, without any significant difference between them, in both seasons. The two mutants of yeast recorded such reduction in acidity percentage than control in the early picking date. Analogical yeast effects on Valencia oranges were reported by Hegab *et al.* (1997), Abdalla *et al.* (1998) and Atawia & El-Desouky (1997) on Washington navel orange.

Concerning ascorbic acid (Vitamin C), Table (5) shows clearly that spraying with mutants (A & B) of yeast resulted in an increment in values of V.C. compared with the control in both seasons. Mutant A gave the highest significant of Vitamin C. It recorded 58.2 & 59.1 mg/100 ml juice in the two seasons, respectively. Mutant B came the next, it reached 52.4 and 54.2 mg/100 ml juice. The control treatment recorded the lowest significant values of vitamin C (46.2 & 493 mg/100 ml juice) in 2001/2002 and 2002/2003 seasons, respectively.

The present findings may be due to the presence of cytokinin precursors in yeast. It was previously proved that some concentration of cytokinin lead to a significant increase in fruit content of ascorbic acid (Bisaria and Rastogi, 1988; Khalil, 1990 and Shehata, 1990).

These results are in harmony with that obtained by El-Emery (2002), who found that ascorbic acid content of tomato fruits was significantly increased as a result of foliar application of yeast (*S. cerevisiae*).

Table (6) : Effect of spraying the two mutants of yeast on some chemical properties on Valencia orange trees during 2001/2002 and 2002/2003 seasons,

Characters / Treatments	T.S.S. (%)				Acidity (%)				L-ascorbic acid (mg/100 ml juice)	
	2001/2002		2002/2003		2001/2002		2002/2003			
	1st harvest time	2nd harvest time	1st harvest time	2nd harvest time	1st harvest time	2nd harvest time	1st harvest time	2nd harvest time	2001/ 2002	2002/ 2003
Control	11.23a*	11.53a	12.0 a	12.00a	1.36a	0.10a	1.37a	0.10a	46.2c	49.3c
Trees sprayed with mutant A	10.77a	11.57a	10.84a	11.43a	1.42a	0.10 a	1.36a	0.10a	58.2a	59.1a
Trees sprayed with mutant B	10.23a	10.73a	10.77a	10.90a	1.27a	0.10 a	1.25a	0.10a	52.4b	54.2b

*Values followed by the same latter in each column are not significantly different at 5 % level according to Duncan's Multiple range test.

Amino acids content in Valencia orange fruits :

Amino acids are an important class of organic compounds and have been heavily researched for their amazing healing potential and powerful contribution and effects on the body's metabolism. Out of the 20 amino acids that make up our bodies, eight is essential and cannot be manufactured by the body and must be ingested in the diet. Therefore, amino acids content in *Valencia orange* fruits were estimated.

Data presented in Table (7) showed the effect of spraying Valencia orange trees with the two mutants (A and B) of *S. cerevisiae* on amino acids content. In general, the amounts of most of them were larger than that of control fruits. A slite increase of amino acid percentages (4.11 % for aspartic acid, 6.38 % for Isoleucine and 6.15 % for lysine) was recorded from trees sprayed with mutant A in comparison with 27.71, 42.86 and 29.89 % in the case of spraying with mutant B. Moreover, hislidine and serine gave highly increase of amino acid percentages (96 %and 96.78 %) after spraying with mutant B, while spraying with mutant A recorded 61.33 and 49.57 % increase, respectively.

Table (7) : Effect of foliar application of two *S. cerevisiae* mutants on amino acid contents of Valencia orange fruits.

Amino acids	Amino acid concentration (*µg*/ml)		
	Untreated trees	Trees sprayed with Mutant (A)	Trees sprayed with Mutant (B)
Alanine	3.12	4.73	4.97
Arginine	8.82	12.22	14.63
Aspartic acid	22.12	23.03	28.25
Glutamic acid	11.06	8.82	3.41
Glycine	1.44	2.10	5.59
Histidine	1.50	2.42	2.94
Isoleucine	3.92	4.17	5.60
Leucine	1.58	2.07	2.38
Lysine	4.55	4.83	5.91
Proline	53.06	110.70	135.31
Serine	4.66	6.97	9.17
Threonine	2.42	3.40	3.71

Glycine showed a great difference between the two treatments (A & B). Trees sprayed with mutant B gave a high level of increase (228 %), while trees sprayed with mutant A gave only 47.14 %. This amino acid facilitates the release of oxygen for the cell-making process, key role in manufacturing of hormones and health of immune system.

In comparison with untreated fruits, a high level of proline content were obtained from both treatments. The percentage of increase reached to 108 and 155 for A and B treatments, respectively. In contrast, glutamic acid content was decreased to 8.82 and 3.41 *ug*/ml for the same treatments, respectively. A significant increase in proline content accompanied by a concomitant decrease in glutamine pool was clearly visible in tobacco plants

(Brugiere *et al.*, 1999). Over expression of genes encoding P5C (`-pyrroline-5-carboyxlate) synthetase, the bifunctional enzyme for catalyzing the conversion of glutamate to P5C, increased proline production and gave osmotolerance to transgenic plants (Hong *et al.*, 2000). Furthermore, overproduction of proline also enhanced root biomass and flower development in transgenic tobacco plants under drought-stress conditions (Kishor *et al.*, 1995).

Conclusively, the present investigation supported the positive effects of foliar spray of *S. cerevisiae* mutants on leaf area (cm^2), N, P, K and Ca levels in leaves , as well as, the reduction of pre-harvest fruit drop percentage. Moreover, treatments enhanced yield (kg), fruit weight, fruit diminutions, improving fruit quality and amino acid contents.

On basis of the obtained results, foliar sprays of yeast mutants are recommended for Valencia orange fruits. Further research on planting Valencia orange trees under drought or osmotic stress conditions is needed and is currently in progress.

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تأثير الرش ببعض طافرات الخميرة على اشجار البرتقال الصيفى

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تعتبر ثمار المواليح من افضل الفواكه المرغوبة بالنسبة للاستهلاك المحلى او بغرض التصدير الى عديد من دول العالم وبخاصة تلك الثمار التى يتم انتاجها باستخدام الاسمدة العضوية والحيوية . لذلك تم تخطيط هذه الدراسة لاختبار قدرة طافرات خميرة ساكارومايسيس سيريفيسيا كمنظم نمو نباتى طبيعى على امكانية تحسين الحالة الغذائية للاشجار وكمية المحصول وخصائص الجودة للثمار وذلك خلال موسمى ٢٠٠١/٢٠٠٢ ، ٢٠٠٢/٢٠٠٣ بمزرعة خاصة بقويسنا بمحافظة المنوفية .

وتشير اهم النتائج المتحصل عليها الى مايلى :

١- عند معاملة خلايا خميرة ساكارومايسيس سيريفيسيا بالمطفر الكيمارى ايثيل ميثان سلفونيت بتركيزات ٢٥ ، ٥٠ ، ١٠٠ ميكرولتر/مل لمدة ٦ ساعات على درجة ٣٠°م بلغت نسبة الحيوية ١٣,٦٢ و ٤,٨ و ٠,٧٥ % على الترتيب .

٢- اظهرت بعض الطافرات زيادة ملحوظة فى معدل تراكم بعض الاحماض الامينية وبخاصة البرولين حيث بلغت نسبة الزيادة فى طافرتين حوالى ١٠ اضعاف السلاله الاصلية بالاضافة الى تحملهما للاسموزية المرتفعة عند تنمية هذه الطافرات فى بيئة تحتوى على امول كلوريد صوديوم وتم انتخاب هاتين الطافرتين وعمل معلق من خلاياهما بتركيز ١ مل(٤ × ١٠ ^٧) خليه لكل لتر لرش اشجار البرتقال الفالنشيا فى منتصف شهر مايو (قطر الثمرة ١,٢ – ١,٨ سم) والاسبوع الرابع من شهر يونيو والاسبوع الاول من شهر اغسطس خلال موسمى الدراسة .

٣- اظهرت النتائج تحسنا معنويا للحالة الغذائية للاشجار وانخفاضا معنويا فى نسبة تساقط الثمار قبل الجمع . كما اوضحت زيادة ملحوظة فى كمية المحصول وخصائص الثمار الطبيعية والكيميائية فى كلا ميعادى الجمع علاوة على زيادة محتوى الثمار من فيتامين سى والاحماض الامينية .

من النتائج المتحصل عليها يمكن ان نوصى برش طافرات خميرة ساكارومايسيس سيريفيسيا بغرض زيادة المحصول وتحسين صفات ثمار البرتقال الفالنشيا .