Effect of Potash Fertilization on the Activity of Isozymes Related to Flowering of Some Sugarcane Varieties

EL- Taweel, Fayza M. A. * , Sahar F. Tawfik and Ouf, A. A*****

* Plant Physiology Department. ** Agricultural Treatments Department.

*** Laboratory of Biotechnology.

Agricultural Research Centre, Sugar Crops Research Institute, Sugar Crops Research Station, Sabahia, Alexandria.

ABSTRACT

The main objective of this investigation was to study the relationship between four levels of potassium sulfate fertilization (0, 24, 48 and 96 Kg/feddan) and four different isozyme systems; namely, Peroxidase, Esterase, Catalase and Amylase, which related to flowering of four sugarcane varieties (GT54-9, F1٠٢, BO19 and CO 413) which were planted at the Experimental Farm of Sabahia Agric. Res. Station at two growing stages: (a) After one month from the different levels of potassium sulfate applications, and (b) Just before flowering. The obtained results demonstrated that there were different activities of the abovementioned four isozyme activities between the two periods of Potash applications. Furthermore, the activities of the four isozymes were superior before the flowering rather than after application of potash fertilization. However, activities of the four isozyme banding patterns have been increased; especially under the highest doses of potash fertilization (96 Kg/feddan) for all the tested varieties. Moreover, flowering percentage was observed for all varieties at the application of high levels of potash fertilization. In general, it could be concluded that increasing in potash fertilization up to 96 Kg/feddan, maximized the activities of different isozyme banding patterns, which relevant or correlated to sugarcane flowering.

INTRODUCTION

Sugar is a strategic commodity in many countries. Owing to the increasing gap between production and consumption of sugar in Egypt, there is an urgent need to conduct several studies to increase sugar yield. The increase of sugarcane could be achieved through the vertical extension, since the horizontal extension is difficult because its requirements for irrigation. The improvement of agricultural practices and development of new desired varieties that characterized with high yield, high sugar content and resistant to major diseases could be considered as methods of vertical extension of sugarcane (Tawfik *et al.*, 1997).

It is believed that sugarcane doses not flower naturally in Egypt. Efforts have been done and high success was met in 1971, when flowering was performed in 18 sugarcane clones under the Egyptian conditions (Rao *et al.*, 1973). Continuous works have been done till recent and flowering is

achieved in almost 150 sugarcane varieties every year at Sugar Crops Research Institute, Agricultural Researc Station Farm, Sabahia, Alexandria (gaber *et al.*, 1992 and Abou El-Fatth *et al.*, 1994). Therefore, great efforts are directed towards the improvement of sugar crop either by using conventional breeding methods (Gaber *et al.*, 1990 and Abu El Fath *et al.*, 1994) or via tissue culture techniques and their applications (Sharaf and Ouf, 1995 a,b; Ouf *et al.*, 1996; Sharaf and Ouf, 1998, 1999; Sharaf *et al.*, 2000 and Ouf *et al.*, 2003).

Furthermore, potash which is absorbed as K^+, is the most abundant cation accumulating in the cell sap of sugarcane. The functions of K in sugarcane are many and have been extensively reviewed by Filho(1985). Among those functions which may be singled out is the main role of K as an enzyme activator in plant metabolisms such as in photosynthesis, protein synthesis, starch formation, and translocation of proteins and sugars. In fact, potash has been reported to control the hydration and osmotic concentrations of stomata guard cells; when K is deficient it causes a loss of turgor pressure resulting in closure of the stomata; reduction in the rate of transpiration; and CO_2 assimilation (Humbert, 1968). For sugarcane grown under moisture stress, application of extra K has been reported to give higher cane and sugar yields (Filho, 1985). In addition, voluminous work on different isozyme systems, which associated to flowering mechanism in sugarcane, was done (Mäkinen and Brewbaker, 1967; Gallacher and Berding, 1995). Furthermore, the effect of potash nutrition on the protein, total nitrogen, and phosphorus concentrations were detected in many plants (Scott 1952, and Besford, 1978).

The main purpose of this investigation was to detect the response of the four different isozyme banding patterns which are highly related to flowering at different potash fertilization level.

MATERIALS AND METHODS

Four varieties of sugarcane (*Saccharum officinarum* L.); namely, GT54-9, F153, BO19, and CO413, were planted on 21th of September, 2006 season at the Experimental Farm Sabahia Research Station. Physical, mechanical, and chemical properties of the experimental soil were analyzed according to the method described by Jackson (1967) and Page (1982) [Table (1)].

The field experiment was assigned in a completely randomized block design (CRBD) with three replicates. The levels of potash fertilizer were 0, 24, 48 and 96 Kg K_2O/ feddan,applied as potassium sulfate (48% K) and the level of nitrogen fertilizer was 50 Kg N/feddan added as urea (46%N). Equal levels of both tested fertilizers were added; the first level was applied

after one month of planting and the second one after one month from the first application. All other cultural treatments of growing sugarcane were carried out as recommended by the Ministry of Agriculture and land Reclamation.

To study the effect of different levels of potash fertilization on the activity of the four isozymes; namely, Peroxidase, Esterase, Catalase and Amylase; one gm of young leaf tissues was crushed into 0.125 M Tris-borate buffer ($PH^{8.9}$), shacked for two hours, and then the suspension was centrifuged at 12000 rpm under cooling for 10 min. The supernatants were used for isozyme assays using Agar starch polyvinnylperilidne (P.V.P) gel electrophoresis according to El-Metainy *et al.* (1977). Analysis of Isozyme patterns was carried out at two subsequent periods: the first period was after one month from applying different levels of potash fertilization, and the second one was just before the flowering stage. Table (2) represents the gel buffers, the electrode buffers, and the staining solutions used for the evaluation of the four isozyme banding patterns.

RESULTS AND DISCUSSION

Effect of potash fertilization levels on the given sozyme banding patterns of the tested sugarcane varieties.

Isozyme banding patterns and their zymograms are shown in figure (1); a description of the peroxidase profile of the four sugarcane varieties after adding the different levels of potash tested fertilization and just before the flowering stage. All varieties in all treatments showed peroxidase isozyming patterns with two anodal bands expressed as A1 and A2, and four cathodal bands expressed as C1, C2, C3 and C4, except for GT54-9 and BO19 varieties in the first treatment (after the addition of potash fertilizer), which did not express the bands C3 and C4, respectively. Interestingly, the first cathodal band was found to be similar for all treated varieties; and such similarity has been changed before flowering. In addition, the second anodal band that appeared after the treatment in the all varities, has not been expressed in case of the examination before the flowering stage. Generally, the activity of peroxidase isoenzymes was superior with increasing potash levels (up to 96 Kg K_2O/ feddan), compared with the low levels of potash fertilization (0, and 24 Kg K_2O /feddan).

Esterase isozyme banding pattern and its zymogram are shown in Figure (2), respectively, for the sugarcane varieties after adding the different levels of potash fertilization and just before flowering. All varieties after adding the various levels of potash fertilization only had one cathodal band (C1) and one anodal, while the collected samples just before

flowering showed two cathodal bands with one anodal band, except for F153 variety that did not express the band A1.

The esterase activities that differed in their electrophoretic mobility, which have been presented in both, are in harmony with those obtained by Ramagopal (1994) and Milfored *et al.*(2000).

Catalase isozyme banding pattern and its zynogramof the same sugarcane varieties were illustrated in Figure (3), in respect; indicated the application of the different levels of potash fertilizer, and before the flowering. All varieties in either case showed three cathodal bands and two anodal bands, except for F153 variety that had four cathodal bands after adding the high level of potash fertilization (96 Kg K_2O/ feddan) and before the flowering process. Similar results were obtained by Dang and Verma (1996); Gulati *et al.*(1998), and FAO, (2001).

The activities of Amylase isozyme patterns and its zymogram for the same sugarcane varieties after the application of the different levels of potash fertilization and just before the flowering stage, respectively, were declared in Figure (4). Expectedly, all varieties showed different activities of amylase isozyme which, rapidly, increased as potassium fertilizer levels increased. On one hand, two and one cathodal and anodal bands, successively, were observed after the application of the various levels of potash fertilization for all sugarcane varieties. On the other hand, tested leaf samples being harvested before flowering were superior in activities of amylase isozyme, with three and two cathodal and anodal bands, in respect, than those which were taken after the application of the different levels of potash fertilization, especially, at highest level of potash fertilization (96 Kg K_2O/ feddan).

The higher percentage of flowering was corresponded to the application of the higher levels of potash fertilization for the tested varieties. Furthermore, var. GT54-9 was superior in flowering percentage compared with the other varieties under study as illustrated in graph (5).

These results were in accordance with those obtained by Fitzpatrick and Guillard (2004); who determined the effects of varying K and N fertilization rates on sugarcane and on six isozyme systems which were related to flowering process; namely, Peroxidase, Esterase, Catalase, amylases, leucine aminopeptidases and acid phosphatases. They also indicated that the total activity of the examined isozymes was, rapidly, increased before flowering. Moreover, the association of different isozyme systems in flowering process was demonstrated in previous researches (Garcia *et al.*, 1991; Chowdhury *et al.*, 1993; Taylor *et al.*, 1995; Kvaratskhlia *et al.*, 1997, Fieldes *et al.*, 1998 and Gallacher & Berding, 2006).

Finally one can concluded that sugarcane producers have to pay more attention to the efficiency of such used fertilizer.

REFERENCES

Abou EL- Fatth, M. F.; A. A. Gaber; Y. H. M. Tawfic and EL Talkhawy, N. M. A. 1994. Efeect of sowing dates on flowering and seed setting of some sugarcane varieties at Alexandria. Egypt. Alex. Sic. Exch. 15 (1):105-125.

Bedford R. t. 2006. Effect of potash nutrition on leaf protein concentrations and growth of young tomato plants. Plant and Soil. 42(2): 441-451.

Chowdhury, MKU, Basil IK. 1993. Molecular analysis of plants regenerated from embryogenic cultures of hybrid sugarcane cultivars (*Saccharum* ssp.). Theoretical and applied genetics; 114: 1237-45.

EL- Metainy, A.Y; A. Y. Abou- youssef; M. I. Sherif; and Sahrigy, M. A. 1977. Isozymes variation in lycopersicon species. Egypt. J. Genet. Cytol. 6(2): 360-369.

Fieldes MA, Gerhardt KE. 1998. Flax guaiacol peroxidases can be used to illustrate the possibility of misinterpreting the effects of stress on the activity of developmentally regulated enzymes. Plant Sci; 132: 89-99.

Fitzpatrick R.J. and Guillard K. 2004. Kentucky Bluegrass Response to Potash and Nitrogen Fertilization. Crop Science 44: 1721-1728.

Gaber, A. A.; Samia S. El- Maghraby; M. H. EL. Deeb; EL Helbawi, F.H. and M. F. Abou EL- Fatth 1990. Correlation between stalk weight and some morphological characters in plant crop and first ratoon of some sugarcane varieties in Alexandria. Annals of Agric. Sic. Moshtohor, 28 (4): 1947-1973.

Garcia-Florenciano E, Calderòn AA, Pedreño MA, Muñoz R, Ros-Barcelò A. 1991. The vacuolar localization of basic isoperoxidases in grapevine suspension cell cultures and its significance in indole-3-acetic acid catabolism. Plant Growth Reg 10: 125-138.

Gallacher, D. J. L. and Berding N. 1995. Use of isozyme phenotypes for rapid discrimination among sugarcane clones. Australian Journal of Agricultural Research. 46(3) 601– 609.

Hammer O.; D.A. Harper, and P.D. Ryan. 1997. Total Lab Program. (TL). Www. Total Lab.com.

Jackson, M. L. 1967. "Soil Chemical Analysis" Constable, Co, Ltd., London.

Kvaratskhelia M, Winkel C, Thorneley RNF. 1997. Purification and characterization of novel class III peroxidase isozyme from tea leaves. Plant Physiol. 114: 1237-1245.

Mäkinen Y. and J.L. Brewbaker. 1976. Isoenzyme Polymorphism in Flowering Plants I. Diffusion of Enzymes Out of Intact Pollen Grains. Physiologic Plantarum.20 (2): 477.

Ouf, A. A.; M. A. Sharaf, and EL- Taweel F. M. A. 1996. An efficient regeneration system for sweet sorghum, *Sorghum Bicolor* L. Monch (var. Tracy). Annals of Agric. Sic. Moshtohor. 34 (1): 1381- 1388.

Ouf, A. A.; Sabra, F. S. and Amal Haussien, A. 2003. Biochemical mechanism of glyphosate in sugarcane (Saccharum officinarum L.) tolerant calli. Pest cont & Environ. Sic. 10 (2): 117-131.

Page, A. I. 1982. "Methods of Soil Analysis" Part 2: Chemical and Microbiological Properties, (2 nd Ed.) Agron. Q: A. S. A., Inc., Madison, Wisc., USA.

Scott E. 1952. Effects of Potash Deficiency on Growth and Metabolism of Sunflower Plants. Botanical Gazette. 114 (2): 165-180.

Sharaf, M. A and A. A. Ouf. (1995 a). High efficient regeneration system of sugarcane (var. GT 54- GT54-9) required for gene transfer. J. Agric. Sic. Mansoura Univ. 20(1): 421-432.

Sharaf, M. A and A. A. Ouf. (1995 b). Embryogenic calli induction and their regeneration in three varieties of sugar beet. J. Agric. Sic Mansoura Univ. 20 (5): 2274-2286.

Sharaf, M. A and A. A. Ouf. 1998. Selection of salt- tolerance mutants from sugarcane calli (var. GT 54- GT54-9). Proceedings of the 26 th Annals meeting of Genetics, Alex. 29-30 Sep, pp 139-147.

Sharaf, M. A and A. A. Ouf. 1999. Micropropagation method of two varieties of sugarcane Saccharum officinarum L. through axillary bud culture. Annals of Agric. Sic. Moshtohor. 37 (4): 2409-2418.

Sharaf, M. A.; A. A. Ouf and El- Maghraby S.S. 2000. Selection of high sucrose-yield (from sclones first ratton) through somaclonal variation of sugarcane Saccharum officinarum L var. GT 54- GT54-9. J. Agric. Sic. Mansoura Univ. 25 (5): 2579- 2588.

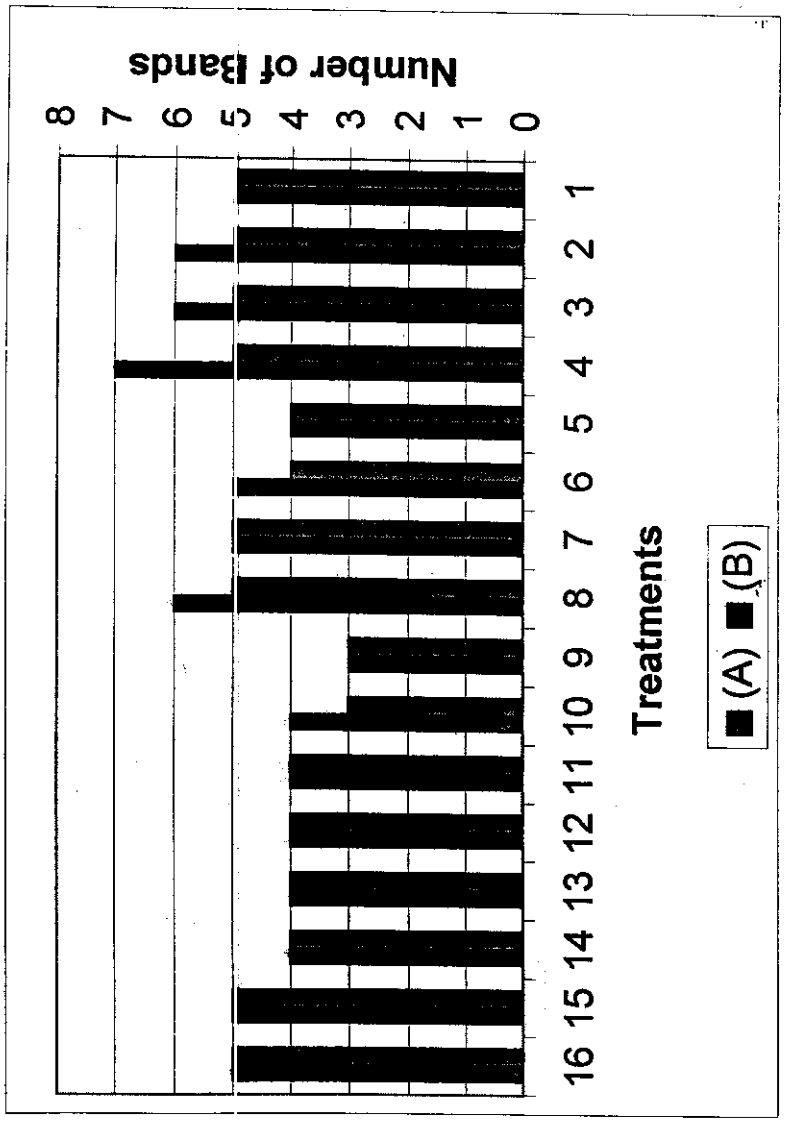
Taylor PWJ, Fraser TA, Ko H-L, Henry J. 1995. RAPD analysis of sugarcane during tissue culture. IN: Terzi M, Cella R, Falavigna A, editors. Current issues in plant molecular and cellular biology. Kluwer Academic Publishers. P.241-246.

(1a)

(1b)

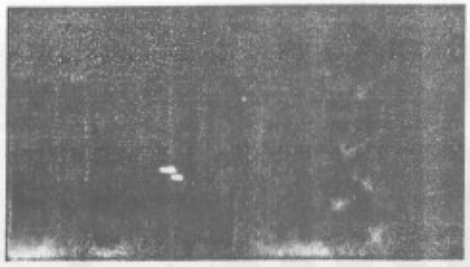
Figure (1a), Peroxidase isozyme banding pattern (Left) and its zymogram (Right) after one month from applied different doses of potash fertilization, (1b) before flowering.

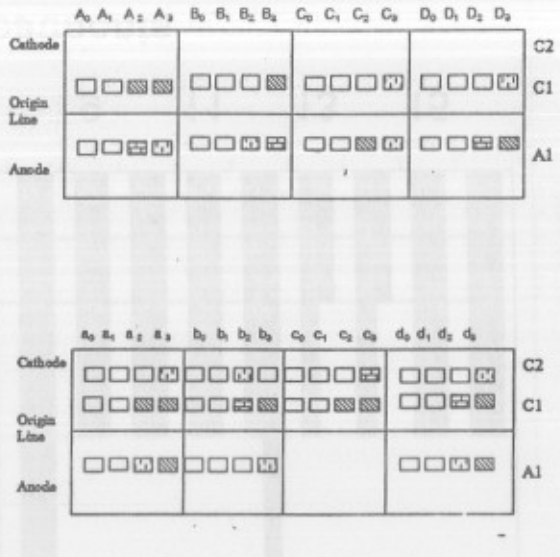
- Band intensities are expressed as follows: ■ ≥20 ▨ ≥15 ▩ ≥10 ▤ ≥5 □ <5
- For zynogram key symbol see Table (4)



Graph (1): Peroxidase isozyme patterns for the four sugar cane varieties as key symbol, see Table (3)
A. after one month from applied different doses of potash fertilization.
B. before flowering.

(2a)

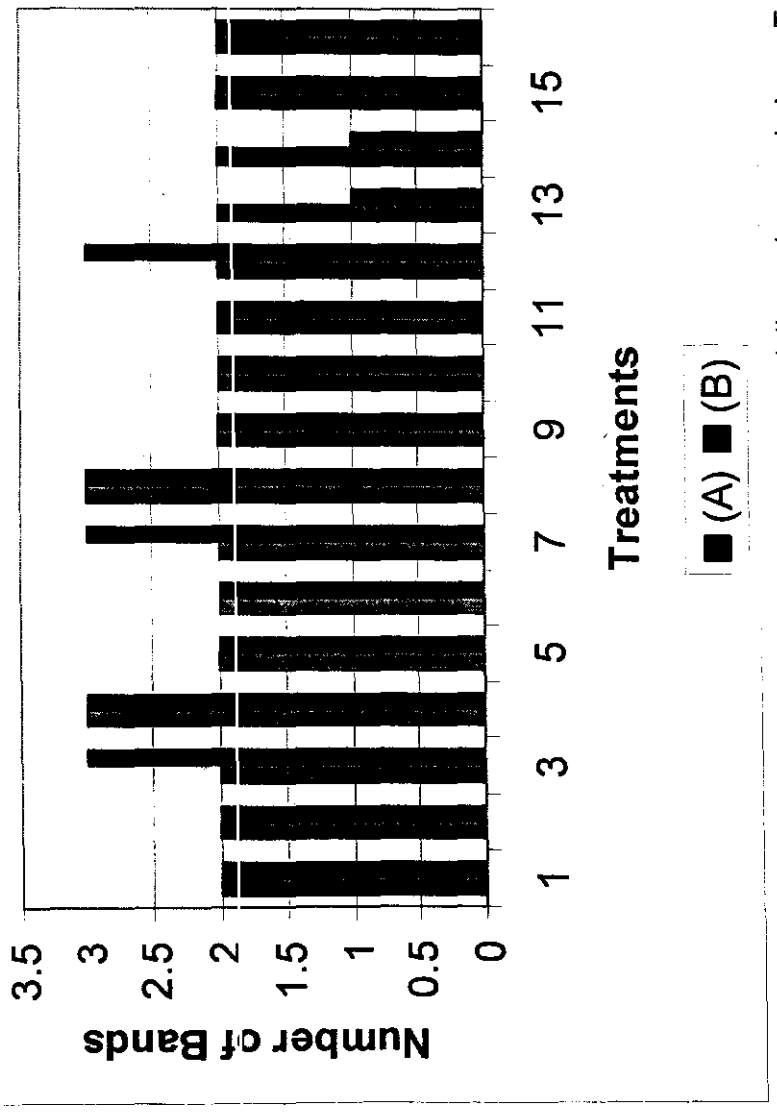




(2b)

Figure (2a), Esterase isozyme banding pattern (Left) and its zymogram (Right) after one month from applied different doses of potash fertilization, (2b) before flowering.

- Band intensities are expressed as follows: ■ ≥20 ▨ ≥15 ▩ ≥10 ▤ ≥5 □ <5
- For zynogram key symbol see Table (4)



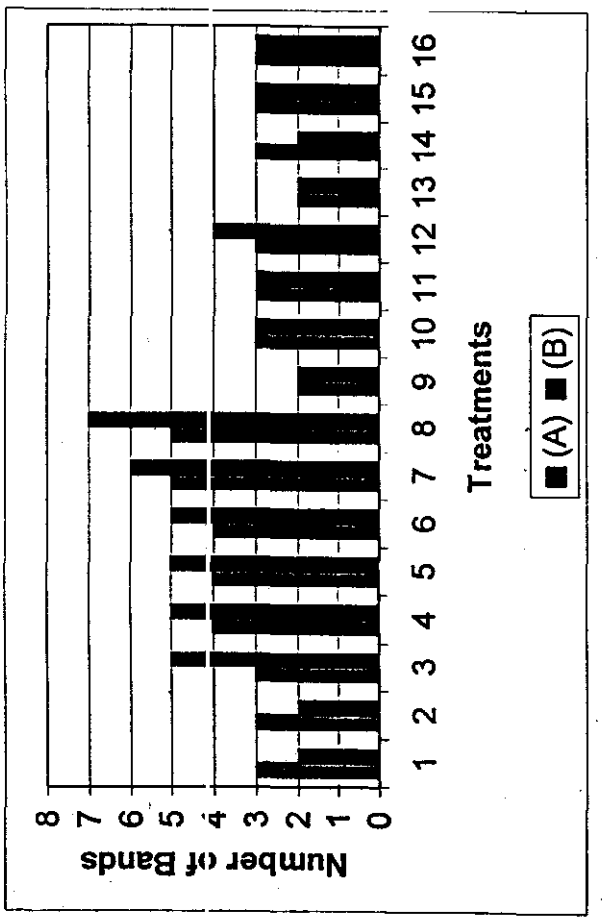
Graph (2): Esterase isozyme patterns for the four sugar cane varieties as key symbol, see Table (3)
A. after one month from applied different doses of potash fertilization.
B. before flowering.

(3a)

(3b)

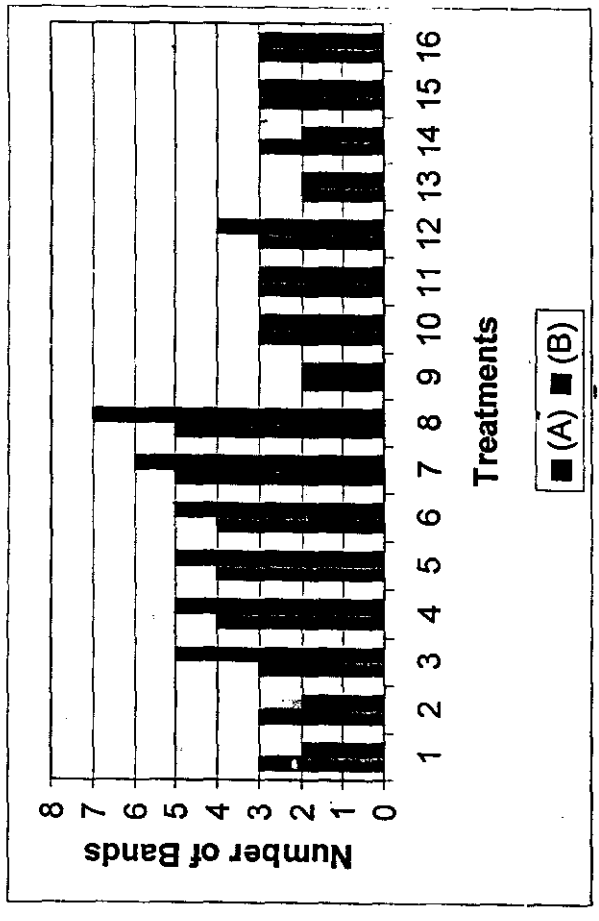
Figure (3a), Catalase isozyme banding pattern (Left) and its zymogram (Right) after one month from applied different doses of potash fertilization, (3b) before flowering.

- Band intensities are expressed as follows: ≥20 ≥15 ≥10 ≥5 <5
- For zynogram key symbol see Table (4)



Graph (3): Catalase isozyme patterns for four sugar cane varieties key symbol, see Table (3)

A. after one month from applied different doses of potash fertilization
B. before flowering



Graph (3): Catalase isozyme patterns for four sugar cane varieties key symbol, see Table (3)

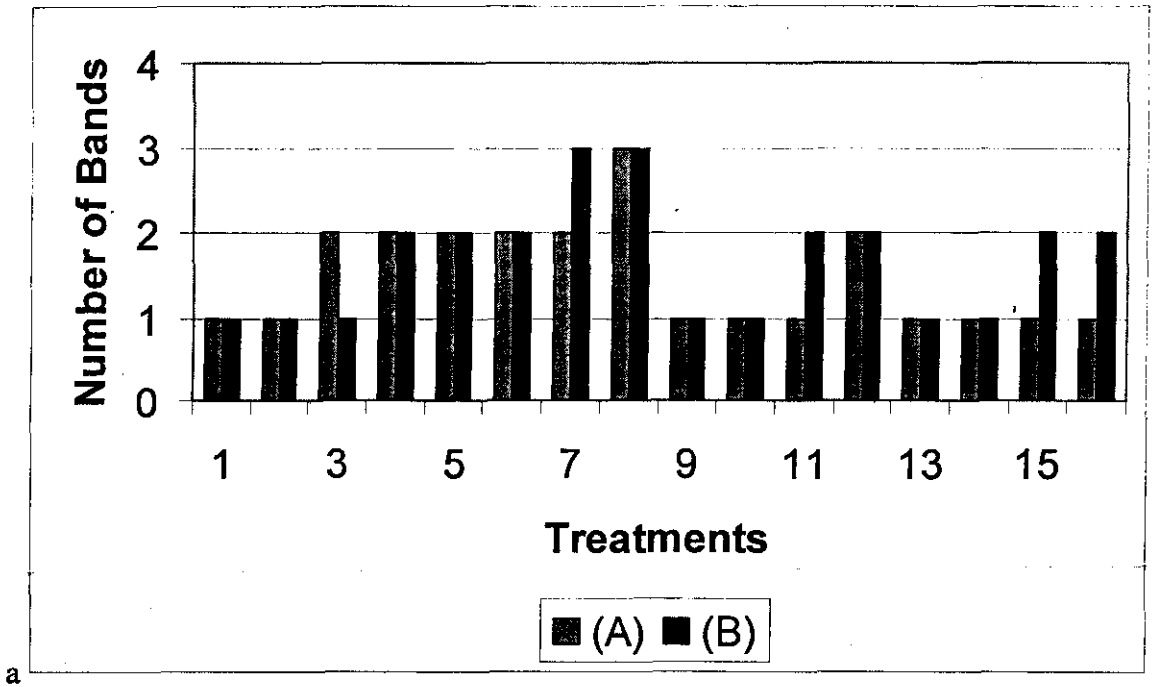
A. after one month from applied different doses of potash fertilization
B. before flowering

(4a)

(4b)

Figure (4a), Amylase isozyme banding pattern (Left) and its zymogram (Right) after one month from applied different doses of potash fertilization, (4b) before flowering.

- Band intensities are expressed as follows: ≥20 ≥15 ≥10 ≥5 <5
- For zynogram key symbol see Table (4)



a

Graph (4): Amylase isozyme patterns for four sugar cane varieties key symbol, see Table (3)

A. after one month from applied different doses of potash fertilization
B. before the flowering

Table (1): Some physical, mechanical and chemical properties of the surface layer (30cm) of the cultivated experimental soil.

Soil Characters		Season (2005)	Season (2006)
Mechanical analysis	Clay	42.30	43.10
	Silt	43.10	42.80
	Sand	14.60	14.10
Texture class		Clay loam	Clay loam
Organic matter (%)		1.38	1.25
PH $_{1:25}$		7.78	8.02
$CaCO_3$ (%)		5.80	6.90
E.C dSm^{-1}		4.75	4.62
Total N (%)		5.80	0.13
Soluble cations (meq.L-1)	Cl^-	27.12	25.00
	K^+	1.25	0.96
	Ca^{++}	17.25	16.31
	Mg^{++}	11.30	10.20
	Na^+	4.60	13.90

Table (2): Gel buffers, electrode buffers and the staining solutions for evaluating the isozyme systems.

Enzyme	Electrode buffer	Gel buffer	Staining
Peroxidase (PRX)	0.3 M Boric acid 0.1 M NaOH PH 8.0	15 mM Tris 3.5 mM Citric acid PH 7.8	0.01 M Sod. Acetate acetic acid PH 5 0.1 gm Benzidine 0.5 ml 0.5% H_2O_2
Esterase (EST)	0.1 M Boric acid 0.1 M NaOH PH 8.6	15 mM Tris 4 mM Citric acid PH 7.8	0.01 M PH osp H ate buffer Ph7.0 + 3 ml substrate (0.25 g β-naphthylacetate + 0.25 g α-naphthylacetate + 50 ml acetone + 12.5 ml H_2O)
Catalase (CAT)	0.1 M Boric acid 0.1 M NaOH PH 8.6	15 mM Tris 4 mM Citric acid PH 7.8	0.5% starch + 0.5% H_2O_2 + 0.5%kl + 0.5ml Acetic Acid Glecial
Amylase (AMA)	0.1 M Boric acid 0.1 M NaOH PH 8.6	15 mM Tris 4 mM Citric acid PH 7.8	1% starch + 0.5% Kl + 0.5ml Acetic Acid Glecial

The obtained data from all varieties for different isozyme systems, regarding the two selected periods under this study, were scanned and compared using Total Lab program (1.11 Version) according to Hammer *et al.*, (1997).

Table (3): Key symbol of Graphs showing patterns of the four isozyme systems.

Pattern number	Variety	Potash fertilization levels
1	GT54-9	**Non- treated (Control)**
2	GT54-9	24 Kg/feddan
3	GT54-9	48 Kg/feddan
4	GT54-9	96 Kg/feddan
5	BO19	**Non- treated (Control)**
6	BO19	24 Kg/feddan
7	BO19	48 Kg/feddan
8	BO19	96 Kg/feddan
9	F153	**Non- treated (Control)**
10	F153	24 Kg/feddan
11	F153	48 Kg/feddan
12	F153	96 Kg/feddan
13	CO413	**Non- treated (Control)**
14	CO413	24 Kg/feddan
15	CO413	48 Kg/feddan
16	CO413	96 Kg/feddan

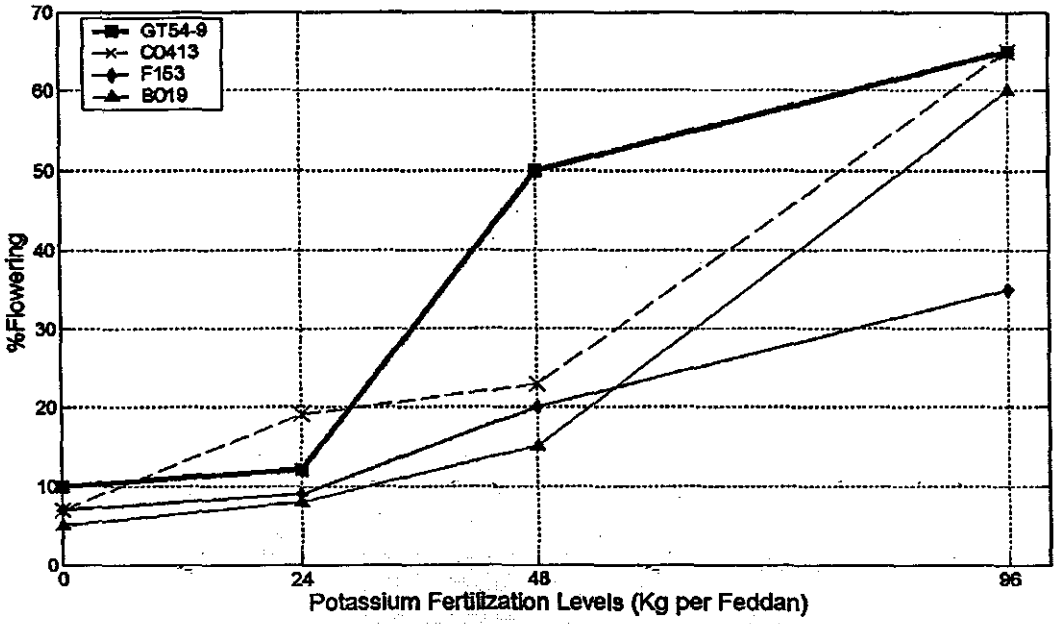
The first experiment after one month of treatment for potash fertilization levels of the tested sugar cane (*Saccharum officinarum* L.) varaieties.

The second experiment just before the flowering of the tested sugar cane (*Saccharum officinarum* L.) varieties.

Table (4): Key symbol of Dendograms showing patterns of the four isozyme systems.

Time of examination	Pattern Symbol	Variety	Potash fertilization levels
After one month of treatment for potash fertilization levels	A0	C9	Non- treated (Control)
	A1	C9	24 Kg/feddan
	A2	C9	48 Kg/feddan
	A3	C9	96 Kg/feddan
	B0	BO19	Non- treated (Control)
	B1	BO19	24 Kg/feddan
	B2	BO19	48 Kg/feddan
	B3	BO19	96 Kg/feddan
	C0	F153	Non- treated (Control)
	C1	F153	24 Kg/feddan
	C2	F153	48 Kg/feddan
	C3	F153	96 Kg/feddan
	D0	C0	Non- treated (Control)
	D1	C0	24 Kg/feddan
	D2	C0	48 Kg/feddan
	D3	C0	96 Kg/feddan

Time of examination	Pattern Symbol	varity	Potash fertilization levels
Before the flowering of four varieties of sugar cane (*Saccharum officinarum* L.)	a0	C9	Non- treated (Control)
	a1	C9	24 Kg/feddan
	a2	C9	48 Kg/feddan
	a3	C9	96 Kg/feddan
	b0	BO19	Non- treated (Control)
	b1	BO19	24 Kg/feddan
	b2	BO19	48 Kg/feddan
	b3	BO19	96 Kg/feddan
	c0	F153	Non- treated (Control)
	c1	F153	24 Kg/feddan
	c2	F153	48 Kg/feddan
	c3	F153	96 Kg/feddan
	d0	C0	Non- treated (Control)
	d1	C0	24 Kg/feddan
	d2	C0	48 Kg/feddan
	d3	C0	96 Kg/feddan



Graph (5): The correlation between potash fertilization levels and percentage of flowering for the different sugarca varieties.

الملخص العربي

تأثير أربع مستويات من التسميد البوتاسى على نشاط مشابهات الإنزيمات المرتبطة بعملية التزهير لأربعة أصناف من قصب السكر

* فايزة ابو الفتوح الطويل ، ** سحر فايز توفيق و *** عاطف احمد عوف

* قسم فسيولوجية النبات ** قسم المعاملات الزراعية *** معمل البيوتكنولوجي

مركز البحوث الزراعية, معهد بحوث المحاصيل السكرية,

محطة بحوث المحاصيل السكرية, الصبحية, الأسكندرية.

يهدف هذا البحث لدراسة تأثير المستويات المختلفة من السماد البوتاسى (٠ ، ٢٤ ، ٤٨، ٩٦ كجم/ فدان) على أربعة إنزيمات مختلفة (البيروكسيديز، الاستيريز، الاميليز، الكتاليز) و التي ترتبط بشدة بعملية الإزهار في أربعة أصناف مختلفة من نبات قصب السكر و هي (GT54-9, F 153, CO413 و BO19) و التي تمت زراعتها بمحطة البحوث الزراعية – الصبحية – الإسكندرية – حيث تم اخذ عينات ورقية لدراسة نشاط الإنزيمات محل الدراسة في فترتين: الأولى بعد مرور شهر من التسميد و الثانية قبل الإزهار مباشرة.

و يمكن تلخيص النتائج المتحصل عليها كمايلى:

١. أنه تم التحقق من وجود اختلاف في نشاط كل من الإنزيمات الأربعة محل الدراسة اختلافا واضحا بين الفترة الأولى و الفترة الثانية لأخذ العينات.
٢. زيادة نشاط للإنزيمات محل الدراسة في الأربعة أصناف التي تم دراستها عند المعاملة بالمستوى الأعلى من السماد البوتاسى (٩٦ كجم/ فدان).
٣. لوحظ أيضا زيادة النسبة المئوية للإزهار بزيادة معدلات إضافة السماد البوتاسى للأصناف الأربعة محل الدراسة و بصفة خاصة فى الصنف GT54-9.

إجمالاً تؤكد هذه الدراسة العلاقة الموجبة بين زيادة مستويات التسميد البوتاسى و زيادة نشاط الإنزيمات التي تساهم في عملية الإزهار لنبات قصب السكر.