Changes in Phenolic Content and peroxidase Activity during the Early Events of *in vitro* Shoot Regeneration in Maize (*Zea mays* L.)

BY

Kamel A. A. H. Tartoura

Agricultural Botany Department, Faculty of Agriculture, Suez Canal University:

e-mail: tartoura@msu.edu

(received, May 3, 2005)

Abstract: *Shoot apex explants excised from 7-day-old maize (Zea mays L.) seedlings germinated in vitro were cultured on MS salt medium containing 500 mg/L casein hydrolysate (CH) and supplemented with various concentrations of 6-benzylaminopurine (BAP) and 2.5 μM, auxin-like-regulator, 2,4-dichlorophenoxyacetic acid (2,4-D), either alone or in combination. Effects of treatments on number of formed shoots, levels of peroxidase (PER) and IAA oxidase (IAA-O) activity, and total soluble phenolic content were investigated during the time-course of shoot induction and early initiation. The present results showed that the number of formed shoots per shoot apex explant was relatively high on medium containing a combination of 2,4-D and BAP as compared to that culture on BAP alone, indicating that BAP containing medium amended with 2,4-D efficiently improved the ability of the explants to form shoots. However, 2,4-D-treated shoot apex explants produced only disorganized callus that poorly regenerated shoots. Results also indicated that activities of PER and IAA-O in BAP-treated explants displayed higher levels relative to either untreated or 2,4-D-treated explants during the progress of shoot initiation. However, the most markedly increase of the occurring enzymatic activities were noted in the combination treatment. In contrast to PER and IAA-O activities, amounts of phenolics in BAP and its combination with 2,4-D displayed a significant transient increase, with the maximum phenolics being at the combined treatment, at around 3 days after application followed by a decrease relative to either untreated or 2,4-D-treated explants that remained relatively high throughout the time-course of experimental analysis. It is concluded that an inverse relationship between soluble phenolics and peroxidase activity is occurred during the time-course of maize shoot regeneration process. Further, it may be also concluded that the transient increase in phenolics might be used as a marker for detecting early stage of morphogenesis*

Abbreviation: AS(F)= adventitious shoot (formation); BAP= 6-benzylaminopurine; 2,4-D= 2,4-Dichlorophenoxyacetic acid; IAA-O= IAA oxidase; PER= peroxidase; PGR(s)= plant growth regulator(s); SE(F)= somatic embryo (formation)

Key words: *In vitro* regeneration, peroxidase, phenolics, *Zea mays*.

INTRODUCTION

Maize (*Zea mays* L.) is widely grown cereal in the world today. Maize is also the most important fodder crop among cereals in industrialized countries and many developing countries. Under the pressures exerted by limited land and water resources, expanding population, and environmental stresses, the great demand for maize of both quality and quantity requires more rapid genetic improvement of maize. Genetically transformed maize plants have been obtained by various approaches, such as particle bombardment (Bohorova *et al.* 1999) and *Agrobacterium* infection (Ishida *et al.* 1996). However, an efficient plant tissue culture procedure with high regeneration frequency is prerequisite for most of the approaches. In maize, Adventitious shoot formation (ASF), i.e. shoot organogenesis (SO), and somatic embryo formation (SEF), i.e. somatic embryogenesis (SE) are the most effective regenerative pathways for obtaining large numbers of plants at a particular developmental stage and many culture systems have been established (Nomura 2003). An *in vitro* regeneration process involves complex interactions between various plant growth regulators (PGRs). Auxin and cytokinin, in particular, play integral roles in the regulation of plant morphogenesis. One of the most fundamental effects of these PGRs is their requirement for cell division both *in vivo* and *in vitro* (Klee and Estelle 1991, Zhang *et al.* 2005). A better understanding of shoot regeneration process is required in order to be able to modify the metabolic pathways in favor of inducing a large number of regenerated plants. Among endogenous factors affecting regeneration, it was reported that phenolics may affect PER and IAA-O activities and in turn modify the level of the natural auxin indole-3-acetic acid (IAA) (Ros Barcelo and Munoz 1992). Any possible correlation between endogenous phenolics, activities of IAA enzymatic oxidation during the early events of the time-course of plant regeneration process are not clear yet.

In this investigation, excised shoot apex explants of germinating maize (*Zea mays* L. cv. Honey N Pearl) seeds were used to study the effects of exogenous application of BAP and/ or 2,4-D on *in vitro* shoot formation. Any possible relationships to endogenous phenolics and enzymatic IAA oxidation during shoot regeneration process were also examined. The overall aim of this work was to study the major physiological changes that may affect shoot regeneration of maize as a model system of cereals.

MATERIALS AND METHODS

Plant material and tissue culture

Mature seeds of sweet-corn (*Z. mays* L. cv. Honey N Pearl) provided by Illinois Foundation Seeds (Champaign, Ill., USA) were surface sterilized with 70% ethanol for 10 min, and 0.1% mercuric chloride ($HgCl_2$) for 15 min. The sterilized seeds were rinsed six times with sterilized water. Sterilized seeds were germinated aseptically on complete MS salt medium (Murashige and Skoog 1962) in 100mm-Petri dishes at 24 ± 2 °C in the dark. A 5 mm long segment of a 7-d-old seedling containing a complete shoot apex and stem proximal to the shoot apex was aseptically excised and cultured on MS basal medium with 3% sucrose containing 500 mg/L CH, 2.5 µM 2,4-D and 2.5, 5, 10, 20 µM BAP, either alone or in combination. This lower concentration of 2,4-D appeared to be the optimal level under the experimental conditions used in this study, giving the highest numbers of shoots in combination with BAP, based on a preliminary experiment. The medium without 2,4-D and BAP served as control. The medium was adjusted to pH 5.5 before autoclaving at 121 °C and solidified with 3 g/L phytagel (Gibco Labs, Grand Island, N.Y., USA). Three shoot apices were cultured for 4 weeks in each Petri dish on 20 ml of the medium by placing them horizontally with the cut edges in close spatial contact with the medium. All plates were sealed using Nescofilm. Incubation condition was identical to those for seedling growth. The number of formed shoots per explant was evaluated after subculture for further 4 weeks on the same MS medium but lacking PGRs (Krishnara and Vasil 1995) under light provided by white fluorescent tubes (24 h, 60 µmol [quanta] m^{-2} s^{-1}). Data are the mean values of three independent experiments, each consisting of 30 shoot apices in three replicates.

Enzymatic activities assay

Tissues originated from shoot apices were collected during specified times from 10 µM BAP ± 2.5 µM 2,4-D as well as untreated tissues. After washing in distilled water, samples were frozen in liquid nitrogen, powdered and stored at -20 °C until use. The frozen powder was homogenized in 0.2 M sodium phosphate buffer, pH 6.8 (fresh tissue: buffer = 1: 4). The buffer contained 1% (w/v) polyvinylpolypyrolidone (PVPP) when used to determine IAA-O activity. The homogenate was centrifuged at 27,000g for 30 min at 4 °C, and the supernatants were used to determine enzymatic activities.

Total soluble PER was assayed according to a modified method of Hammerschmidt *et al.* (1982). The assay mixture in a total volume of 3 ml contained 10 mM K-phosphate buffer, pH 7.5 at 25 °C, 2 mM H_2O_2 and 9 mM guaiacol as the substrate. After addition of 5 µL of crude enzyme extract, increase in absorbance was measured at 470 nm by a Beckman DU 530 spectrophtometer at intervals of 30s up to 2 min. PER activity was expressed as enzyme units min^{-1} g^{-1} FW.

IAA-O activity, estimated by H_2O_2 independent oxidation of IAA, was measured spectrophotometrically according to a modified method of Beffa *et al.* (1990). The reaction mixture (1 ml) contained 0.5 mM IAA, 0.05 mM $MnCl_2$ and 150 mM sodium acetate buffer, pH 4.6. The enzymatic reaction was initiated by adding 2 µl of enzyme extract. After incubation for 20 min at room temperature (25 ± 2 °C) in the dark, the non-oxidized IAA was complexed by the addition of 2 ml of a $FeCl_2$-H_2So_4 reagent consisting of 15 ml of 0.5 M $FeCl_2$, 50 ml H_2O and 300 ml of 36 M H_2SO_4. The unstable red chelated dye was quantified after 15 min at 530 nm at the point of maximal dye intensity. The concentrations of IAA were quantified using IAA standard curve and the amounts of oxidized IAA were calculated by subtracting the remaining IAA from the total IAA amount. Data are the mean values of three different extracts. IAA-O activity was expressed as µg IAA oxidized 20 $min^{-1}g^{-1}$ FW.

Total soluble phenolic content determination

About 500 mg FW from the same bulk of frozen powder was homogenized in 2.5 ml 80% methanol (MeOH). The homogenized sample was incubated for 15 min at 70 °C and then filtered on a funnel; the residue on the filter was washed with 2.5 ml 80% MeOH to optimize the extraction. Final volume was adjusted to 5 ml and used immediately for soluble phenolics. The total amount of phenolics was determined spectrophotometrically at 760 nm using Folin-Ciocalteau reagent (Dai *et al.* 1994). The calibration curve was constructed using chlorogenic acid. Phenolic concentration was expressed as µg of total soluble phenolics g^{-1} FW. All data are the mean values of three different extracts.

All chemicals used in this investigation including the basal MS medium were obtained from Sigma Chemical Co., St. Louis, Mo., USA.

RESULTS

Shoot formation

Fig. 1 shows that untreated shoot apices did not differentiate into shoots since undifferentiated cells senesced after 4 weeks. Similarly 2,4-D-treated shoot apices did not produce shoots. However, in one of 9 replicates, just a single piece of callus cells regenerated one shoot only. On contrast, BAP-treated shoot apices markedly increased the number of formed shoots/ explant, with the maximum effect being at 10 μM. However, the combination of 2,4-D and BAP treatments significantly produced higher number of formed shoots, with the maximum effect being at 10 μM BAP + 2.5 μM 2,4-D, compared with BAP alone. It is evident that BAP synergistically acted with 2,4-D in stimulating shoot apices to produce shoots.

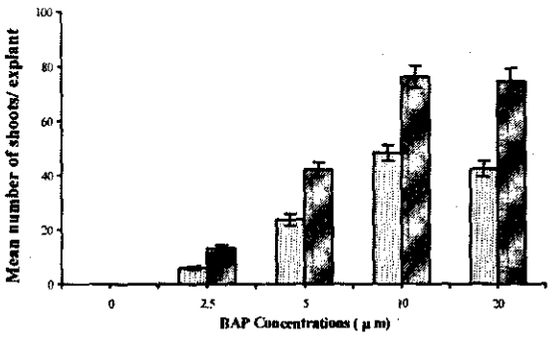

Fig. 1. Number of formed shoots by shoot apices of *Zea mays* cultured in lightness for 4 weeks in basal MS medium after a 4-week pretreatment in darkness with either 2.5 μM 2,4-D or BAP at various concentrations either individually or in combination. All values are means of 3 independent experiments, each consisting of 30 shoot apices. Vertical bars represents ± SD. □ , BAP; ■ , BAP + 2.5 μM 2,4-D.

However, 2,4-D-treated shoot apices poorly underwent morphogenesis and senesced after 4 weeks, as also did the control cultures. In the light of the obtained results, it may be concluded that BAP-containing medium supplemented with 2,4-D improve the efficiency of shoot formation of maize under this experimental system.

Light microscopic studies were conducted to ascertain the route of *in vitro* maize differentiation in response to BAP ± 2,4-D. Microscopic observations revealed that presence of meristematic zones in the shoot apical region of both BAP ± 2,4-D. In shoot apices grown on medium containing BAP alone, the meristematic cells underwent divisions and developed into well-defined shoot primordia. Each shoot primodium had a meristematic dome shaped apical meristem protected by a leaf primodium. These numerous shoot primordia further underwent *de novo* differentiation and produced multiple shoots and these had well defined vascular connections with the parent tissue, indicating that some *de novo* differentiation of shoot apices observed in the present work were actually the result of ABF. However, it was also observed that meristematic zones present in the shoot apices grown on medium containing combination of BAP plus 2,4-D developed into formation of somatic embryos as well as adventitious buds, depending on BAP concentration. Somatic embryos had no vascular connection with the parent tissue, indicating that some of the formed shoots were actually the result of somatic embryos germination.

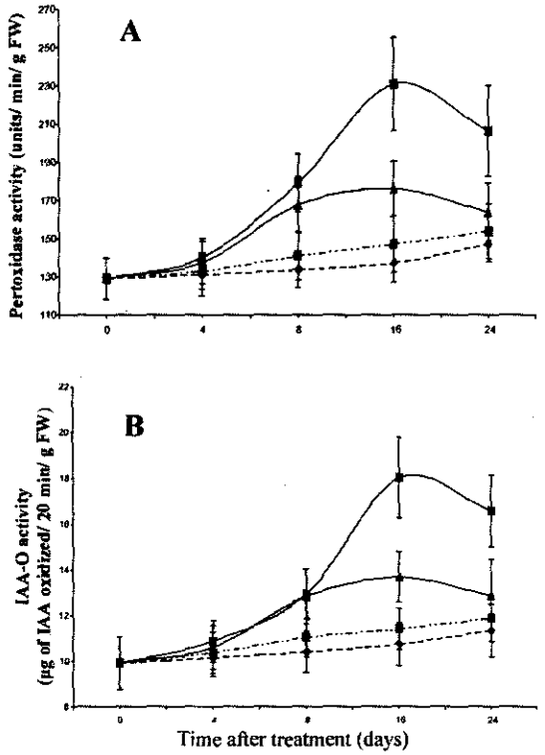

Fig 2. The time-course of the changes in total soluble PER (A) and total soluble IAA-O (B) activity levels in excised shoot apices of Z. mays after BAP ± 2.5-D application during the early events of either cellular dedifferentiation in the case of callus derived from untreated and 2,4-D-treated shoot apices or cellular shoot differentiation in the case of formed shoots in the rest of the treatments. All values are means of three different extracts. Vertical bars represent ± SD --◆-- , control; --■-- , 2.5 μM 2,4-D;—▲— , 10 μM BAP; —■—, 2.5 μM 2,4-D + 10 μM BAP

Enzymatic activities and phenolic content

Figure 2A Shows that PER activity increased gradually during the early events of shoot formation in response to BAP ± 2,4-D application, with the maximum effect being at the combined treatment, relative to either control or 2,4-D, indicating a positive correlation between increased peroxidase activity and the number of formed shoots (Figs. 1 and 2A). It was evident that PER activities significantly increased in proliferation tissues originated from shoot apices which might be differentiated into shoots and their levels positively related to the number of formed shoots, as shown in Figures 1 and 2A. Similar trends to those observed in PER activity were also found in terms of IAA-O activity (Fig. 2B). However, in IAA-O assay, it was necessary to add PVPP in the buffer extraction and without it; no IAA-O activities could be detected.

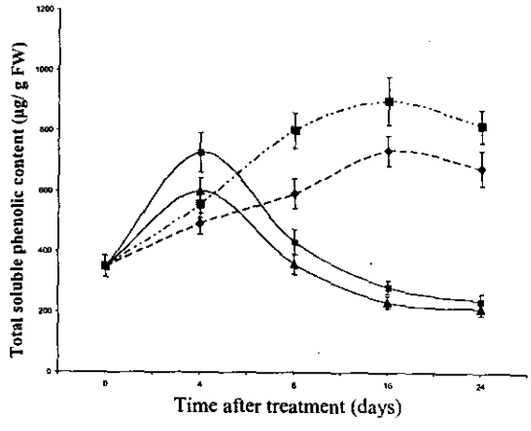

Fig 3.The time course of the changes in total soluble phenolic levels in excised shoot apices of Z. mays after BAP ± 2.5-D application during the early events of either dedifferentiation in the case of callus derived from untreated and 2,4-D-treatment or cellular shoot differentiation in the case of formed shoots in the rest of the treatments. All values are means of three different extracts. Vertical bars represent ± SD. The symbols designations are the same as those in Figure 2.

Since IAA-O activity could not be determined without addition of PVPP in the crude extracts, it was important to examine the concentration of phenolic content during the early events of shoot initiation. Regarding this concept, Figure 3 shows that total soluble phenolics transiently increased at around 3 days after culture in response to BAP ± 2,4-D application, relative to either control or 2,4-D. Thereafter, phenolics gradually decreased with the progress of shoot formation.

In response to 2,4-D application, it was obvious that a higher level of phenolic content was observed during most of the time-course evaluated, as also did the control, suggesting that the gradual decrease in soluble phenolics might be associated with the shoot apical meristem differentiation. Decreasing the level of phenolics after accumulating (Fig. 3) might be explained by the fact that PER and IAA-O are involved in their metabolic utilization since their levels were actually increased during this time (Figs. 2 A and B). Thus, it may be concluded that the stimulatory effect of PGRs used in this study on the initiation of shoots might be explained by their influence on phenolic synthesis, their enhanced accumulation and metabolic utilization during the progress of shoot formation. Further, it may be also suggested that the transient increase in phenolics can be used as a marker for detecting the early stages of morphogenesis.

Discussion

Many plant somatic cells are totipotent, and undergo *in vitro* regeneration via one of two pathways, i.e. adventitious shoots or somatic embryo formation (Thorpe 1994). In this study, *Z. mays* might be regenerated via both morphogenetic pathways from shoot apices excised from seedlings grown *in vitro*, since the medium contained the key regulator for morphogenesis BAP, and the inducer for somatic embryogenesis 2,4-D. These findings are in agreement with Wang (1987), Zhong *et al.* (1992, 1998), and Sairam *et al.* (2003) who reported that BAP alone induced ASF, but BAP plus 2,4-D induced either AS or SE or simultaneous organization of both morphogenesis depending on BAP level. Description of scanning electron microscopic analysis of adventitious shoot formation and somatic embryogenesis from *in vitro* cultured shoot apices of this genotype of maize was illustrated by Zhong *et al.* (1992) who reported that the number of formation of adventitious shoots and somatic embryos was dependent on the size of enlarged meristematic domes with larger ones giving rise to greater numbers of regenerants. In fact, auxins, in particular 2,4-D, are known to promote inducing somatic embryogenesis, whereas embryos development proceeds on medium lacking PGRs (Pasternak *et al.* 2002). On the other hand, cytokinins, in particular BAP, stimulate cellular division by accelerating the cell transition from the G2 phase (the growth phase following DNA replication) to the M phase (mitosis) of the cell cycle (Redig *et al.* 1996, Laureys *et al.* 1998, Stals and Inzé 2001, Zhang *et al.* 2005). These authors also reported that both auxin and cytokinin act at multiple levels affecting transcription of cell cycle genes and/ or the activity of the cyclin-dependent kinases. In addition, their altered balance seems to be required at specific points of the cell cycle. For these reasons, the medium containing 2,4-D alone failed to induce shoots (Fig. 1), indicating the importance of the synthetic cytokinin BAP in maize

morphogenetic response. This concept was previously confirmed by Zhong *et al.* (1992), Bhaskaran and Smith (1990), Krishnara and Vasil (1995) who found that 2,4-D alone did not often induce *in vitro* shoot regeneration in monocot plants and medium supplemented with a proper concentration of a cytokinin induced formation of shoots via AS or SE. To date, it is still not fully understood why somatic embryogenesis is induced in some cases while in others adventitious shoot formation is triggered (Zhang *et al.* 1998). However, since a single cell is able to develop normally into a whole plant via both pathways (Bajaj 1989, Vasil and Thorpe 1994); thus, a cell might contain the developmental program for formation of shoot and somatic embryo formation, but the exact nature of the factor(s) triggering a specific *in vitro* developmental program, whether physical, biochemical and/ or genetic, is still unknown (Zhang *et al.* 1998). Any progress in the identification of genes critical to cell division, shoot regeneration and response to PGRs might provide critical tools in understanding *in vitro* morphogenic responses, as also suggested by Zhong *et al.* (1998), and Roudier *et al.* (2003)

PER and IAA-O activities are involved in many important physiological functions. They might be involved in the breakdown of H_2O_2, catabolism of auxin and many phenolic acids, cross-linking of phenolic components and lignin biogenesis in the cell wall (Edreva 1996). Regarding shoot differentiation and regeneration, PER and IAA-O have been shown to be markers of the initiation of meristematic activity, as also suggested by Kay and Basile (1987). These enzymes may act on phenylpropanoids in the synthesis of lignin or they may mediate cross-linking of polysaccharides or extensions of cell walls, and hence serve an important role in cell wall construction and in the regulation of cell wall plasticity. Interaction between PER and IAA-O activities during growth and differentiation of plant cells is complicated by the fact that the participation of these oxidative enzymes in the regulation of these processes is not only limited by their ability to oxidize auxins and phenols, but also by their participation in various aspects in plant development (Brownleader *et al.* 2000). In fact, IAA oxidase activity has been involved in the metabolic oxidation of IAA in many plant tissues. However, this activity has been associated in most cases with PER activity (Srivastava and Van Huystee 1973, Kieliszewska-Rokicka 1980, Van Huystee 1987).

According to Christianson and Warnick (1985), three phases of shoot regeneration can be distinguished: (a) formation of cell competence; (b) shoot induction and (c) shoot initiation and development. During induction, explants perceive the exogenous cytokinin and auxin compounds and become committed to the development of shoots. Consequently, it seems likely that, in this study, PER and IAA-O activities are involved in shoot differentiation and regeneration rather than shoot induction (Fig. 2). Thus, it may be concluded that PER and IAA-O activities could serve an important role(s) in cell wall construction and in the cell wall plasticity during shoot differentiation, as also suggested by Brownleader *et al.* (2000).

Phenolics are known to be potent modifiers of PER and IAA-O activities. Since wall-associated PERs are suggested to control the endogenous auxin level in the phase of promotion of meristematic activity (Gaspar *et al.* 1985), phenolics may be indirectly involved in the regulation of physiological processes controlled by auxin. The higher levels of soluble phenolics were temporarily elevated in BAP alone or in combination with 2,4-D during the early events of shoot initiation (Fig. 3), presumably prior to any cellular division, followed by gradual decreases during the progress of shoot formation and this reduction might be coincided with the actual beginning of shoot differentiation and *de novo* differentiation for further shoot initiation. During the secondary events of shoot initiation, phenolic content was characterized by a drop in its level while PER and IAA-O activities increased (Fig. 2 and 3). However, soluble phenolics was not characterized by a markedly lower level after accumulating in untreated and 2,4-D treatment, indicating that the transient increase followed by a decrease in BAP ± 2,4-D is marker for successful *in vitro* regeneration. These results demonstrate that the developmental changes produced in treated-shoot apical meristem are due to, at least in part, to the changes of oxidative enzymes and phenolic content. In accordance with the data presented here, a lot of works demonstrated that during shoot regeneration, changes in the level of PER, IAA-O as well as phenolic content were also noted (McDougall *et al.* 1992, Arezki *et al.* 2001, Kanmegne and Omokolo 2003). Based on the forgoing results, a relation between PER and IAA-O activities and phenol metabolism may be part of a more extensive regulatory system for regulating *in vitro* regeneration.

Conclusions

The present results indicate that 2,4-D acts synergistically with BAP in stimulating more shoot formation in shoot apex explants compared to untreated or 2,4-D-treated ones. In addition, an inverse relationship between total soluble phenolics and oxidative enzymes activities, peroxidase and IAA oxidase, during the time-course of shoot formation was found. The stimulatory effect of PGRs used in this study on the initiation of shoots might be explained by their influence on phenolic synthesis, their enhanced accumulation and metabolic utilization during the progress of shoot formation.

Further, it may be also concluded that the transient increase in phenolics can be used as a marker for detecting the early stages of morphogenesis. Further work will be required to fully elucidate the mode of 2,4-D and BAP action in regulating *in vitro* differentiation of adventitious shoots and somatic embryos in *Z. mays* shoot apex explants.

REFERENCES

Arezki O., Boxus P., Kevers C., and Gaspar T. 2001. Changes in peroxidase activity, and level of phenolic compounds during light-induced plantlet regeneration from *Eucalyptus camaldulensis* Dehn. nodes *in vitro*. Plant Growth Regulation 33: 215-219.

Bajaj Y. B. S. 1989. Biotechnology in agriculture and forestry 8: plant protoplasts and genetic engineering 1. Springer, Berlin Heidelberg New York

Beffa R., Martin H. V., and Pilet P.-E. 1990. *In vitro* oxidation of indoleacetic acid by soluble auxin-oxidase and peroxidases from maize roots. Plant Physiol. 94: 485-491.

Bhaskaran S., and Smith R. H. 1990. Regeneration in cereal tissue culture: a review. Crop Sci. 30: 1328-1336.

Bohorova N. E., Zhang W., Julstrum P., Mclean S., Luna B., Briton R. M., Diaz L., Ramos M. E., Estanol P., Pacheco M., Salgado M., and Hoistington D. A. 1999. Production of transgenic tropical maize with *crylAb* and *crylAc* genes via microprojectile bombardment of immature embryos. Theor. Appl. Genet. 99: 437-444.

Brownleader M. D., Hopkins J., Mobasheri A., Dey P. M., Jackson P., and Trevan M. 2000. Role of extension peroxidase in tomato (*Lycopersicon esculentum* Mill.) seedling growth. Planta 210: 668-676.

Christianson M. L., and Warnick D. A. 1985. Competence and determination in the process of *in vitro* shoot organogenesis. Dev. Biol. 95: 288-293.

Dai G. H., Andary C., Cosson L. M., and Boubals D. 1994. Polyphenols and resistance of grapevines (*Vitis* spp.) to downymildew (*Plasmopara viticola*). Acta Hortic. 381: 763-766

Edreva A. 1996. Plant peroxidases. Biochemistry and physiology. J Plant Physiol. 22: 102-104.

Gaspar T., Penel C., Castillo F. J., and Greppin H. 1985. A two-step control of basic and anionic peroxidase and its significance for growth and development. Physiol. Plant. 64: 418-423.

Hammerschmidt R., Nuckles E. M., and Kuc J. 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol. Plant Pathol. 20: 73: 73-82.

Ishida Y., Satto H., Ohta S., Hiei Y., Komari T., Kumashiro T. 1996. High efficiency transformation of maize (*zea mays* L.) mediated by *Agrobacterium tumefaciens*. Nat. Biotechnol. 14: 745-750.

Kanmegne G., and Omokolo N. D. 2003. Changes in phenol content and peroxidase activity during *in vitro* organogenesis in *Xanthosoma sagittifolium* L. Plant Growth Regulation 40: 53-57.

Kay L. E., and Basile D. V. 1987. Specific peroxidases isoenzymes are correlated with organogenesis. Plant Physiol. 84: 99-105.

Kieliszewska-Rokicka B. 1980. Isozymes of peroxidase, indole-3-acetic acid oxidase and polyphenoloxidase of poplar and pine. Acta Physiol. Plant. 2: 195-207.

Klee H., and Estelle M. 1991. Molecular genetic approaches to plant hormone biology. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42: 529-551.

Krishnara J. S., and Vasil I. K. 1995. Somatic embryogenesis in herbaceous monocots. In: *In Vitro* Embryogenesis in plants, Thorpe T. A. (ed.). 417-470 Kluwer, Dordrecht.

Laureys F., Dewitte W., Witters E., Van Montagn M., Inze D., and Van Onckelen H. 1998. Zeatin is indispensable for the G2-M transition in tobacco BY-2 cells. FEBS Lett. 426: 29-32.

McDougall G. J., Davidson D., and Millam S. 1992. Alterations in surface-associated peroxidases during callus development and shoot formation in explants of *linum usitatissimum*. J. Plant Physiol. 140: 195-200.

Murashige T., and Skoog F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Nomura K. 2003. Long-term conservation of embryogenic competence by induction and disorganization of somatic embryos in carrot. Plant Breeding 122: 343-346.

Pasternak T. P., Prinsen E., Ayaydin F., Miskolczi P., Potters G., and Asard H. 2002. The role of auxin, pH, and stress in the activation of embryogenic cell division in leaf protoplast-derived cells of alfalfa. Plant Physiol. 129: 1807-1819.

Redig P., Shaul O., Inze D., Van Montagu M., Van Onckelen H. 1996. Levels of endogenous cytokinins, indole-3-acetic acid and abscisic acid during the cell cycle of synchronized tobacco BY-2 cells. FEBS Lett. 391: 175-180.

Ros Barcelo A., and Munoz R. 1992. Peroxidases: their role in the control of plant cell growth. In: Plant Peroxidase 1980- 1990. Penel C., Gaspar T., and Greppin H. (eds.) Topics and Detailed literature on Molecular, Biochemical, And Physiological Aspects. Univ. of Geneve, Switzerland, pp. 71-89.

Roudier F., Fedorova E., Lebris M., Lecomte P., Györgyey J., Vaubert D., Horvath G., Abad P., Kondorosi A., and Koudoros E. 2003. The *Medicago* species A_2-type cyclin is auxin regulated and involved in meristem formation but dispensable for endoreduplication-associated developmental programs. Plant Physiol. 131: 1091-1103.

Sairam R. V., Parani M., Franklin Z., Lifeng Z., Smith B., MacDougall J., Wilber C., Sheikhi H., Kashikar N., Meeker K., Al-Abed D., Berry K., Vierling R., and Goldman S. L. 2003. Shoot meristem: an ideal explant for *Zea mays* L. transformation. Genome 46: 323-329.

Srivastava O. P., and Van Huystee R. B. 1973. Evidence for close association of peroxidase, polyphenol oxidase, and IAA oxidase isozymes of peanut suspension culture medium. Can. J. Bota. 51: 2207-2215.

Stals H., and Inzé D. 2001. When plant cell decide to divide. Trends Plant Sci. 6: 359-364.

Thorpe T. A. 1994. Morphogenesis and regeneration. In: Plant Cell and Tissue Culture, Vasil I K., Thorpe T. A. (eds). Kluwer Academic Publishers, Dordrecht, pp 17-36.

Van Huystee R. B. 1987. Some molecular aspects of plant peroxidase biosynthetic studies. Ann Rev. Plant Physiol. 38: 205-219.

Vasil I. K., and Thorpe T. A. (eds.) 1994. Plant tissue culture- theory and applications. Kluwer Scientific Publishers, Dordrecht.

Wang A. 1987. Callus and Plant regeneration from maize mature embryos. Plant cell Rep. 6: 360-362.

Zhang K., Diederich L., and John C. P. L. 2005. The cytokinin requirement for cell division in cultured *Nicotiana plumbaginifolia* cells can be satisfied by yeast cdc25 protein tyrosine phosphatase. Implications for mechanisms of cytokinin response and plant development. Plant Physiol. 137: 308-316.

Zhang S., Williams-Carrier R., Jackson D., and Lemaus P. G. 1998. Expression of CDC2Zm and KNOTTD1 during *in-vitro* axillary shoot meristem proliferation and adventitious shoot meristem formation in maize (*Zea mays* L.) and barley (*Hordeum vulgare* L.) Planta 204: 542-549.

Zhong H., Wang W., and Sticklen M. B. 1998. *In vitro* morphogenesis of Sorghum bicolor (L.) Moench: Efficient plant regeneration from shoot apices. J. Plant Physiol. 153: 719-726.

Zhong H., Srinivasan C., and Sticklen M. B. 1992. *In-vitro* morphogenesis of corn *(Zea mays* L.). Differentiation of multiple-shoot clumps and somatic embryos from shoot tips. Planta 187: 483-489.

التغيرات فى محتوى الفينولات ونشاط البيروكسيديز أثناء الأحداث الأولية لإعادة تكوين سيقان الذرة معملياً

كامل أحمد حسين طرطورة
قسم النبات - كلية الزراعة – جامعة قناة السويس

أجرى هذا البحث لدراسة تأثيرات المركب الأوكسينى 2،4 - دايكلوروفينوكسي حمض الخليك بتركيز 2.5 ميكرومولر، المركب السيتوكينينى 6- بنزيل أمينو بيورين بتركيزات 5، 20 ميكرومولر- كلا على حدة أو خليط منهما- فى وجود محللات الكازين بتركيز 500 ملليجرام فى اللتر- على العدد المتكون من سيقان الذرة، مستويات أنشطة انزيمات البيروكسيديز واكسيديز اندول-3-حمض الخليك، أخيرا محتوى الفينولات الكلية الذائبة أثناء حث القمم النامية للذرة على تكوين السيقان منها فى مراحل تكوينها المبكرة. أوضحت النتائج المتحصل عليها أن متوسط عدد السيقان المتكونة لكل قمة كان عاليا فى البيئات المحتوية على خليط من المنظمين بالمقارنة بتلك المنزرعة على بيئات محتوية على كل منظم على حدة ويدل ذلك على أن إمداد البيئة المحتوية على المركب السيتوكينينى بمنظم النمو الأوكسينى كان له تأثيرا فعالا فى تحسين قدرة القمم النامية على تكوين عدد أكثر من السيقان. تشير النتائج المتحصل عليها أيضا أن هناك علاقة إيجابية بين مستويات أنشطة انزيمات البيروكسيديز وأكسيديز اندول-3-حمض الخليك نتيجة المعاملة من جهة وقدرة القمم فسيولوجيا على تكوين سيقان جديدة منها. على عكس الزيادة التدريجية لأنشطة إنزيمات البيروكسيديز وأكسيديز اندول-3-حمض الخليك يتضح أن مستويات الفينولات الذائبة الكلية قد انخفض بعد إرتفاعة إرتفاعا مؤقتا مشيرا إلى إستهلاكة أيضيا أثناء نشأة السيقان. يستنتج من تلك الدراسة أن هناك علاقة عكسية بين الفينولات الذائبة الكلية وأنشطة إنزيمات أكسدة الأوكسينات والفينولات أثناء المراحل المبكرة لعملية تكوين السيقان. أضافة إلى ذلك يمكن أيضا استنتاج أن الزيادة المؤقتة فى المحتوى الفينولى يمكن استخدامها كعلامة لإكتشاف التكوين المورفولوجى فى مراحلة المبكرة.