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LUCIGENIN-AND LUMINOL-ENHANCED CHEMILUMINESCENCE IN MECHANICAL INJURED ROOTS OF CORN (*Zea mays* L.)

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SUMMARY

The chemiluminescence (CL) of roots from corn (*Zea mays* L.) seedling , after mechanical injury , has been observed by lucigenin – or luminol dependent chemiluminescence (LCDC) and (LMDC) respectively . The light emission in injured roots system was blocked by NaCN in LMDC system, whereas ascorbic acid addition resulted about 50% and 70% reduction in light emission in LMDC - and LCDC systems respectively.

In another experiment of injured roots extracts , the Light emission was increased by the addition of H_2O_2 in LCDC and LMDC systems , whereas NaCN addition has blocked effect in (LMDC) system.

The suggested results in this study shows the effect of CL substrates , such as luminol or lucigenin and the addition compounds (NaCN or ascorbic acid radical scavenger) on the CL intensity , may be proved the participation of reactive oxygen species, mainly superoxide anion , and the free radical mechanism in the CL - injured roots reaction of corn plant seedlings .

INTRODUCTION

Plant cells are able to produce reactive oxygen species (ROS) , mainly superoxide radical or H_2O_2 during wounded cells or infection by pathogens . They are formed through the lipid peroxidation (exogenous mechanism) or through the leakage from the electron transport chain (endogenous) mechanism (Mittler , 2002) .

Lucigenin-and Luminol- enhance chemiluminescence are widely used to monitor the formation of ROS in plant and other systems (Russo-Carbolante et al,2003; Khand *et al.* , 2002 ; Lazim , 2001 ; Murphy *et al* . ,1998 ; Bolwell *et al.* , 1998 and Faulkner and Fridovich,1993).

Some hypotheses were proposed from earlier CL studies of seedling, focusing on CL inhibitors and wounded or extracts tissue (Salin *et al.* ,1985 and

NaCN inhibitor were examined by addition of 10µl NaCN to a reaction vessel containing injured root segments , thereafter , CL signal was examined by injection 0.2 ml luminol (Fig.1b).The effect of radical scavengers on peak CL of injured roots was made by addition 0.2 ml ascorbic acid to the reaction vessel containing injured root segments , thereafter , CL signal was examined by injection 0.2 ml luminol or 0.5 ml lucigenin (Fig.1c) and (Fig.2b) respectively. All
CL signals represented one experiment out from triplicate experiments.

In another experiment to examine the CL signal from injured corn roots extracts , 0.2 ml of luminol or 0.5ml of lucigenin were directly injected in to the reaction vessel containing 20µl of roots extract and buffer (Fig.3a) and (Fig.4a) respectively. 0.5 ml $H_2 O_2$ was added to a reaction vessel containing root extracts and buffer , thereafter , CL signal was noted by injection of 0.2 ml luminol or 0.5 ml of lucigenin (Fig.3b) and (Fig.4b) respectively . NaCN inhibitor were examined by addition of 10 µl NaCN to a reaction vessel containing root extracts and buffer ,thereafter , CL signal was examined by injection of 0.2 ml luminol (Fig.3c) . All CL signals represented one experiment out from triplicate experiments.

RESULTS AND DISCUSSION

Injured roots-induced chemiluminescence:

It is well known enhanced CL is detected in whole plant tissue after mechanical injury (Henry
et al . 2004 ; Chen et al . 2003 ; Lida et al . 2000 ; Suzuki et al . 1991 ; Slain et al . 1985 and Slain and Bridges , 1981). Focusing on the injury of a seedlings , root segments from corn plant was examined by CL after mechanical injury . The time course curve for light production increased immediately following the addition of luminol or lucigenin and subsequently decayed to the back ground level and remains constant for about 25 min and 8 min (Fig.1a) and (Fig.2a) respectively.

The reactive oxygen species , such as superoxide anion are produced via a peroxidase enzyme system (Murphy *et al.* 1989). Interaction of the generated oxygenating gents with certain substrates , resulted in the formation of electronically excited oxygenation products. As these products return to their ground state photon emission is responsible for the phenomenon of CL . This CL can be amplified by addition of luminescent substrates, such as luminol and lucigenin (Schepetkin , 1999) resulting in a sensitive tool for examing the oxidative activity of peroxidase system . The light production by injured corn roots may be lead to the destruction of the cell wall and membrane was caused by the promotion of the reaction and contact of CL substances , such as peroxidase , hydrogen peroxide , superoxide anion , and unknown endogenous cellular substances , moreover , the chemiluminescence enhancement can be related to the acceleration of one

Colli *et al.* ,1955). Abeles *et al.* (1978) and Salin and Bridges (1981) suggested that a peroxidase enzyme system contribute to the production of CL - excited species through an animal leukocyte-like system.

Recently,Henry et al (2004)studied the wound-induced CL from plant leaves,and also observed the emission of photons from excited chlorophyll molecules following transfer of leaves to dark . Lida et al (2000) studied the CL of extracts and mechanical injuries from various organs of leguminous seedlings , based on the reactive oxygen species / hydrogen donors mediator screening system , and also demonstrated that levels of CL are of different intensities in different parts of the plant , the lowest being the stem , and the highest in the primary leaves .

The aim of the present study was to examine the ability of injured corn roots and there extracts to form the reactive superoxide radical by means of lucigenin and luminol – dependent chemiluminescence systems .

MATERIALS & METHODS:

Reagents and Chemicals Preparation :

Luminol stock solution (5 – amino – 2 - , 3 - dihydrophazine -1 , 4 – dione) in concentration of 1.13×10^{-3} M was used and prepared according to the modified method of Ewetz and Thore (1976).

Other chemicals and reagents concentrations used were as follow : Lucigenin (0.5 M) , K- Phosphate (0.05 M) , H_2O_2 (32.6×10^{-2}M) , NaCN (1 mM) and ascorbic acid (100 μ M).

Procedure:

Seeds of corn (*Zea may* L.) were soaked in distilled water for 10 h . The seeds then were planted in vermiculite and grown in the dark for 5 days at 25 ^{0}C , then the etiolated seedlings were harvested . Root sections (5 mm in length) were cut n the light from etiolated corn plants , thereafter , mechanical injuries of roots segments were performed in horizontal cuts at two points on the roots.

A crude extract from corn roots were prepared after mechanical injury according to proce-dure of Salin and Bridges (1981) , with slight modification.

CL measurements:

Measurement of the emission light (CL) were made at room temperature in a multipurpose photon counting system type of 9635 QB , designed by Al -Hashimi and Mohammed (1997) , and CL signal was registered on the chart recorder.

To measure the CL signal of injured corn roots , 0.2 ml of luminol or 0.5 ml of lucigenin were injected in to the reaction vessel containing injured root segments in 20 ml K- phosphate(Fig.1a) and (Fig.2a) respectively.

or more steps in the peroxidase- catalyzed reaction prior to the light emission reaction (Abeles *et al* .1978 , and
Salin and Bridges (1981) .

The effect of metabolic inhibitor NaCN on luminol - dependent CL of injured corn roots is shown in Fig.1b . As shown , the inhibitors studied NaCN was caused a rapid inhibition of CL in LMDC-injured roots system.

It is well known that NaCN is one of the most effective inhibitors of peroxidases , depending on this fact , I expected that the inhibition of CL may be occurs through the peroxidase enzyme contributed production of CL- excited species through the injured root tissue (Abeles *et al.* 1978).

The effect of radical scavenger , such as ascorbic acid on luminol – and lucigenin dependent CL of injured corn roots were observed in (Fig. 1c) and (Fig.2b) respectively. As shown , ascorbic acid diminished light emission about 50% and 70% in LMDC and LCDC – injured roots system respectively .

The mechanism of injured roots CL which discussed above may be involves the production of NADPH which react with a NADPH oxidase and O_2 which then could be used in the peroxidation of phenolics compounds to generate increased quantities activated O_2 , such as superoxide anion $^{\cdot}O_2$, and in addition to phenolics , other compounds might be released and could be subject to the action of peroxidases (Abeles *et al* . 1978)

It appears from the results presented in (Fig.1a) and (Fig.2a) that the mechanism of injured corn roots CL may be involves the production activated O_2 species which interact with externally supplied

Extract roots – induced chemiluminescence:

It is well known that the extracts of whole plant tissue produced CL during the process of germination (Leda et al.2000 ; Watanabe et al. ,1991 and Warm and Laties , 1982). Focusing on the extracts of a seedling , extracts from corn injured root seedlings was examined for CL in LMDC and LCDC systems (Fig. 3a) and (Fig.4a) respectively .

The level of light emitted by root extracts , might be due to some CL compounds involved in root parts which synthesized during the germination phase, such as flavonid , phenolic acid and simple phenols , which are contributed to a mechanism of CL – excited state formation.

Light emission in LMDC – and LCDC –root extracts was increased upon the addition of H_2O_2 (Fig.3b) and (Fig.4b) respectively , while NaCN addition leads to decreases in the CL in LMDC –root extracts system (Fig.3c).

According to Abeles et al. , 1978 , the light emission in corn root extracts might be showed the occurrence of the greatest a mount of peroxidase involve in the mechanism of CL emission , which correlated well with finding that root , moreover , the increasing in the CL by the addition of H_2O_2 was leading to the fact that H_2O_2 may be rat – limiting in corn roots extracts – induced CL.

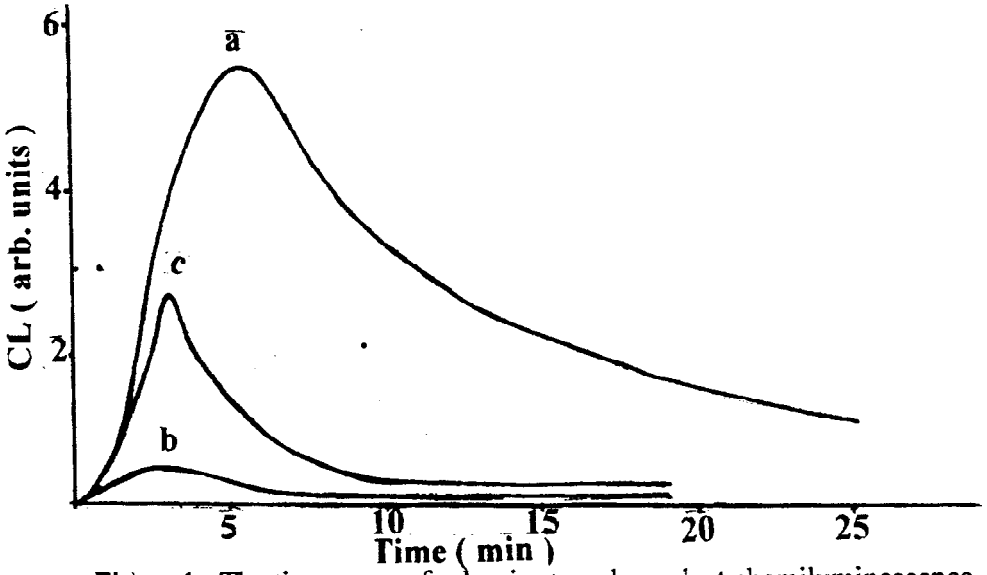

Figure 1 . The time course for luminol – dependent chemiluminescence of injured roots from corn plant seedlings as a control curve (a) . Curves (b) and (c) represents the effect of addition NaCN and ascorbic acid on a control curve respectively.Experimental details are given in procedure.

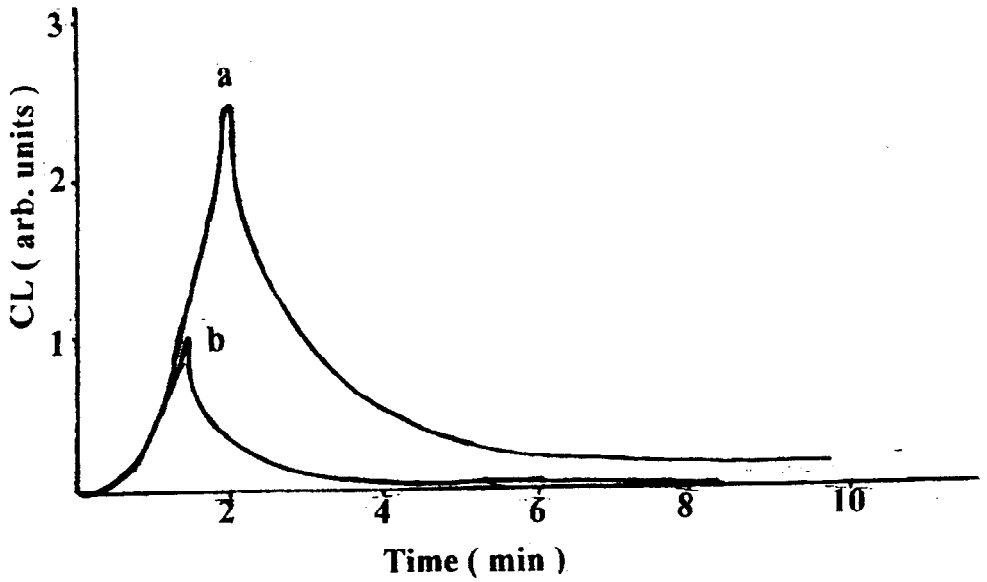

Figure 2 . The time course for lucigenin – dependent chemiluminescence of injured roots from corn plant seedlings as a control curve(a).Curve(b) represent the effect of addition ascorbic acid on a control curve. Experimental details are given in procedure .

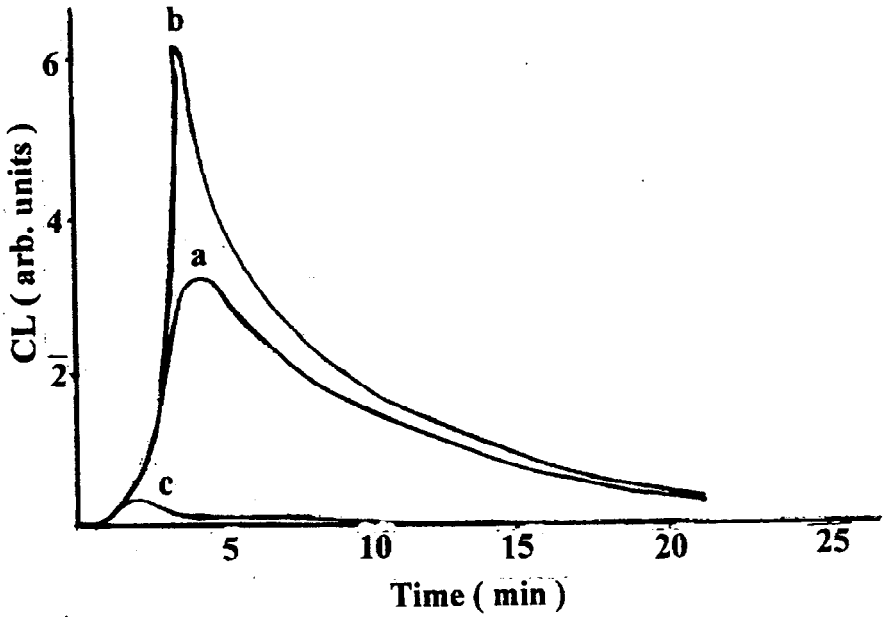

Figure 3.The time course for luminol-dependent chemiluminescence of extracts from corn of injured roots seedlings as a control curve (a). Curves (b) and (c) represents the effect of addition H_2O_2 and NaCN on a control curve respectively. Experimental details are given in procedure.

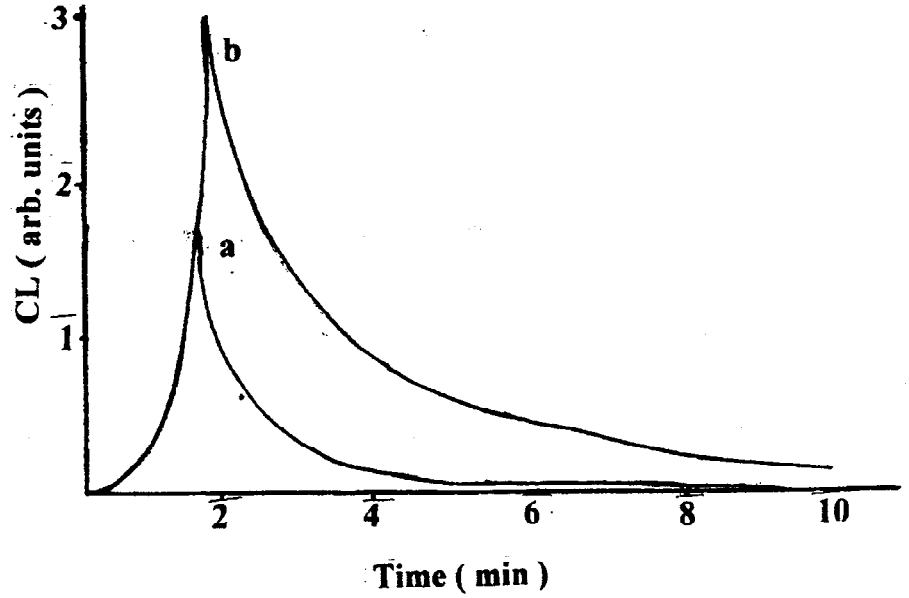

Figure 4. The time course for lucigenin-dependent chemiluminescence of extracts from corn of injured roots seedlings as a control curve(a). Curve(b) represent the effect of addition H_2O_2 on a control curve. Experimental details are given in procedure.

According to Thorpe and Kricka (1986) , the mechanism of the peroxi-dase – catalyzed CL oxidation of luminol may be involves a formation of a complex between oxidation (H_2O_2) and peroxidase to produce a luminol radical. Luminol radicals then undergo further reaction resulting in the formation of endoperoxide , which decomposes to yieled an electronically excited 3 – aminophath- alate dianion emitting light on returning to its ground state .

Lucigenin CL has been described as s specific for O_2 (Corbisier et al. , 1987),and the classical reaction scheme of lucigenin interaction with O_2 resulting in the formation of unstable dioxetane which decomposes forming two molecules of methylacridone and emittion light quanta (Schepetkin , 1999) .

The mechanism of lucigenin dependent CL may be requires an initial reduction followed by the reaction with superoxide .The superoxide radical can itself accomplish the initial reduction , the reduced form then reacting with an addition superoxide molecule . The reduction of lucigenin is sufficient to give a positive signal , the reduced lucigenin can reduce oxygen and the resulting supero- xide reacts with additional reduced lucigenin to give CL (Liochev and Fridovich (1997). As noted by these mechanism , the superoxide radical is essential to the CL whether it is formed by the action of peroxidase which might be involve in root extracts, moreover , the ability of peroxidases to elicit CL from lucigenin under condition in which these peroxidases are known to produce superoxide shows that the reduction of lucigenin is sufficient to give CL .

Finally , the ROS are produced in both unstressed and stressed cells , in addition of , the plants have well– developed defense systems against ROS , involving both limiting the formation of ROS as well as instituting its removal (Alscher et al , 2002) . As well as , the CL emission in response to stress-particularly oxidative stress is a common source of damage cells in plants and animals systems (might play a signaling role in inducing defenses to pathogens) , and results from increased production of ROS (Henry et al. , 2004).

In conclusion , further experiments in LMDC or LCDC method would be needed to determine the levels of oxygen radicals or the oxidative burst , when the roots plant is exposed to an oxidizing or reducing agent . In addition to examining the CL of wounded plants as a result of lipid peroxidation reactions , which act as signals to induced plant defense responses .

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التألق الكيميائي المحسن باللوسيجينين و اللومينول للجذور المستحدثة ميكانيكيا لنباتات الذرة الصفراء

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الخلاصة

تم مشاهدة التألق الكيميائي لجذور نباتات الذرة الصفراء المستحدثة ميكانيكيا// وذلك باستخدام محلول اللوسيجينين واللومينول . كان لتأثير إضافة سيانيد الصوديوم على الجذور المتضررة تأثيرا مثبطا على التألق الكيميائي عند اللومينول , بينما أحدث إضافة حامض الأسكوربيك نقصان في التألق الكيميائي بحدود 50% و 70% عند اللومينول واللوسيجينين على التوالي .

في تجربة مستخلصات الجذور المستحدثة ميكانيكيا// للذرة الصفراء أحدث إضافة بيروكسيد الهيدروجين على مستخلصات الجذور زيادة في التألق الكيميائي لكلا المحلولين ، بينما أحدث إضافة سيانيد الصوديوم نقصان في التألق الكيميائي عند اللومينول .

النتائج المقترحة لتلك الدراسة تشير على أن تأثير محسنات التألق الكيميائي مثل اللومينول واللوسيجينين وكذلك تأثير المثبط سيانيد الصوديوم وكاسح الجذور حامض الأسكوربيك ، على شدة التألق الكيميائي ربما تؤكد على مشاركة الجذر الأوكسيجيني الحر في ميكانيكية تفاعل التألق الكيميائي لجذور الذرة الصفراء.