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Callus Induction and Shoot Formation for Mexican Red Bean (*Phaseolus vulgaris* L.) Pinto Cultivar *in Vitro*

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Abstract

The current study aimed to adopt a method for inducing callus cells and regenerating the important common red bean using different types of growth regulators such as N^6-benzylaminopurine (BAP), Naphthalene acetic acid (NAA), and Thidiazuron (TDZ). Different types of common bean pinto cultivar explants, such as internodes, cotyledons and roots, were inoculated on Murashige and Skoog medium (MS) provided with different combinations of plant growth regulators, including 1- BAP (5 mg/l) 2-BAP (4.5 mg/l) NAA (0.5 mg/l), 3- BAP (4.5 mg/l), and TDZ (0.1mg/l). Callus was initiated on MS culture medium supplied with 5 mg/l BAP for all explants (internodes, cotyledons, and roots) at 50, 20, and 10% respectively, while adding NAA with 0.5mg/l showed a low percentage of callus (30%) only in the internode explants. Optimum results were obtained by growing the internodes on MS medium with 4.5 mg/l BA and either 0.5 mg/l NAA or 0.1 mg/l TDZ, transplanting the derived shoots into internodes and cotyledons with 70 and 10% respectively. This study concludes that the internodes as explants have the best growth results.

Keywords: *Phaseolus vulgaris* L., red bean Pinto, callus induction, shoots induction, *in vitro*.

استحثاث الكالس والنموات الخضرية للفاصوليا المكسيكية الحمراء المرقطة (*Phaseolus vulgaris* L.) خارج الجسم الحي

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الخلاصه

هدفت الدراسة الحالية اعتماد طريقةلاستحثاث خلايا الكالس ونشوء النموات الخضريةمنالفاصولياء الحمراءباستخدام انواع مختلفة من منظمات النمو وهي–N6 بينزيلامينوبيورين (BAP) ونفثالين استيك اسد (NAA) ثيديازورون (TDZ). استخدمتأنواعمختلفةمنالاجزاء الخضريةللصنفبينتو من الفاصوليا الحمراء المرقطة الشائعة مثل السلاميات،الفلق والجذور وزرعت هذه الاجزاء فياالوسط الزرعي موراشجوسكوغمزودة بمجموعةمختلفةمنمنظماتنموالنبات،وهي 1–BAP (5 ملغم/لتر), 2– 4.5 BAP ملغم/لتر و 0.5 NAA ملغم/لتر,3–4.5 BAP ملغم/لتر، 0.5 ملغم/لتر NAA وTDZ 0.1 ملغم/لتر. استحث الكالس على وسط موراشج وسكوج مزود ب 5 ملغم/لتر N6 BAP لكل جزء نباتي (السلاميات،الاوراق والجذور) وبنسبة 50، 20 او10% على التوالي،بينما عند إضافة 0.5ملغم/لتر من NAA فقط السلاميات انتجت خلايا الكالس

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بنسبة منخفضة 30%. تم الحصول على أفضل النتائج لانتاج المجموع الخضري في الاوساط الزرعية المزودة ب TDZ بتركيز 0,1ملغم/لتر للاجزاء الخضرية (السلاميات و الفلق) بنسبة 70و10% على التوالي. ويتبين من هذه الدراسة ان السلاميات كاجزاء نباتية كانت هي الافضل من بقية الاجزاء الاخرى.

Introduction

Mexico bean (*Phaseolus vulgaris* L.) Pinto cultivar is an economically important crop that is considered as one of the main cultivated legumes for human consumption in Latin America, Africa, and Asia. It represents a substantial source of protein, minerals such as iron and zinc, and particular vitamins [1]. Beans presented with quick bread is regarded typically as a fundamental meal wherever meat is unobtainable, with their amino acids are considered as a full source of protein. Studies reported that pinto beans can lower the bad and good cholesterol levels (LDL and HDL) [2]. Pinto beans contain the phytoestrogen coumestrol that has an assortment of health impacts. They are the most popular bean in North American countries. The nomenclature refers to their mottled skin. The young pods may be harvested and cooked as green pinto beans [3]. However, bean plant growth and production of seed are bounded by treatment with high ratios of chemical products (e.g. fertilizers, herbicides, pesticides or insecticide), as well as drought and nutritional deficiencies [4]. Both Plant biotechnology and conventional breeding methods give a wide domain for bean improvement [5]. Tissue culture is turning to be an alternative vegetative propagation approach of plants. *In vitro* growing of plants typically prevents microorganism and flora diseases. Virus demolition and maintenance of plants in a virus-free condition can even be speedily achieved in cultures. The three main methods generally used in tissue culture are the micropropagation through the enhanced multiplication of axillary bud, organogenesis, and somatic embryogenesis [6]. In *in vitro* cultures, the majority of protocols of common bean regeneration are relied on either the direct regeneration or shoot initiation from different explants, such as apical shoots and roots, cotyledonary nodes, internodes, petioles, etc [7, 8]. Although *in vitro* indirect regeneration has been achieved, few studies reported the indirect regeneration of *P. vulgaris* and the regeneration of the shoot from callus is quite little, which highly depends on cultivars [9, 10]. Thus this study aimed to the *in vitro* regeneration of red bean in Iraq. An efficient and reproducible protocol of the regeneration was achieved through organogenesis from cotyledonary nodes that were arisen from seedlings.

Materials and Methods

Medium preparation

MS medium ready- made from Himedia Company was used and then supplemented with sucrose [11]. By using 0.1 N of NaOH or N HCI, the pH of the medium was adjusted to 5.8 and then 4 g/l agar was added to the medium. The volume of the medium was completed to 1 liter. The medium was dissolved by heating on a hot plate with magnetic stirrer till boiling. An aliquot of 10 ml of the medium was dispensed into universal tubes which were autoclaved at 121°C under pressure of 1.04 kg/cm for 15min. Cultures were incubated at 22 ± 2ºC under 16 h light+8h dark photoperiod.

Sterilization of seed and preparation of explants

Firstly, seeds of common bean were washed with running tap water and then rinsed two times with sterile deionized water. Surface sterilization was performed by immersion of seeds for 1 min in ethanol (70%), which were then immersed for 10 or 20 min in 3 or 6% of NaOCl, respectively. Drops of Tween 20 were added and the seeds were rinsed four times in sterile deionized water and left in sterile water overnight. Thereafter, seeds were inoculated on MS medium for germination [11]. Cultures were kept at 22 ± 2 ºC under dark, then the explants were excised from the 7-11 day seedling.

Callus induction and shoot formation

Explants of red pinto bean (internodes, cotyledon, and roots) were cultured on MS medium provided with different combinations of plant growth regulators, 1- BA (5 mg/l) 2- BA 4.5mg/l and 0.5 mg/l NAA 3- BA 4.5mg/l,0.5mg/l NAA and 0.1mg/l TDZ. All cultures were incubated at 22 ± 2ºC with a 16 h light+8h dark cycle. Five replicates of each treatment were used. After three weeks of incubation, the percentage of callus induction was measured using the equation:

Callus induction frequency= (No. of explants initiated callus / total number of cultured explants) x 100.

Shoot induction frequency= (No. of shoot per each explants / total number of explants) x100 [12, 13].

Results and Discussion

Effect of sodium hypochlorite solutions at different concentrations and application periods

Effects of sodium hypochlorite solutions (NaOCl) at different concentrations and periods were examined. The results showed that the seed germination, growth of seedling, and tissue viability decreased noticeably by NaOCl at high concentrations, while at low concentrations. Also, the ability of regeneration of the tissue is influenced in a negative manner by higher concentrations and longer treatment periods with disinfectants , but they showed maximum effect against microbiological contamination [14,15].The process of sterilization under *in vitro* conditions should aim to permanently using the disinfectant at low concentrations for the shortest time [16,17]. This study aimed to evaluate the effects of NaOCl solutions utilized for sterilization on the *in vitro* seed germination and seedling growth of *P. vulgaris* L.. The optimum results were obtained using 3% NaOCl for 10 min for all examined parameters. Seed- borne contamination was increased gradually by decreasing the concentration and time period of NaOCl exposure to below 6 and 10 min. Remarkable decreases were recorded at 6% NaOCl in all cases, which may be attributed to the harmful effects on the seed embryo. Seed germination was decreased to 0% when NaOCl concentration was increased to 6% at 20 min period of time. Faster growth was observed in seedlings that are grown from seeds sterilized with 3% NaOCl for 10 min as compared to those sterilized with other concentrations and time periods Figures-(1, 2). The height was decreased with the increase of the concentration to 6% X [12].

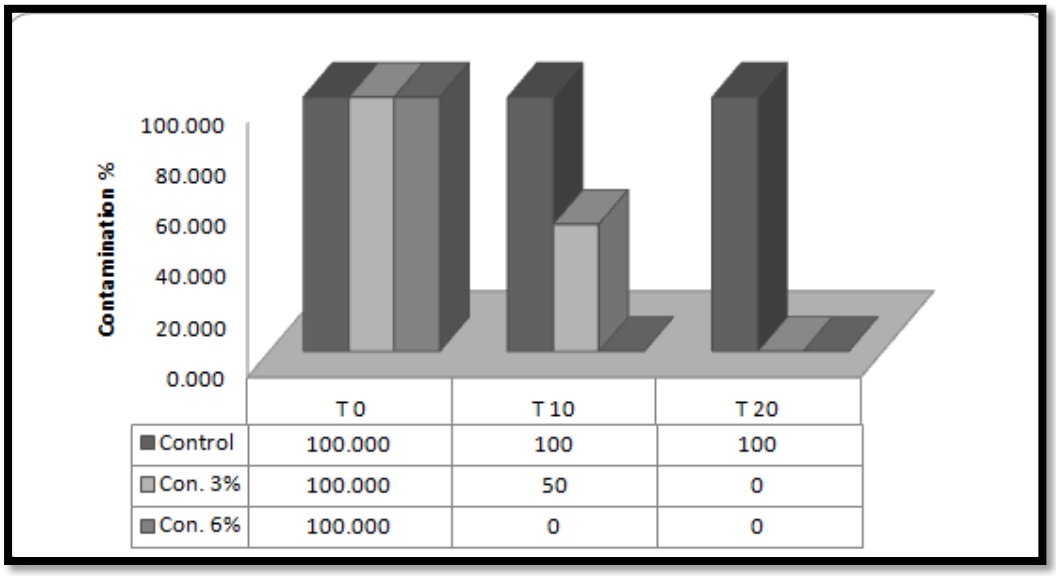
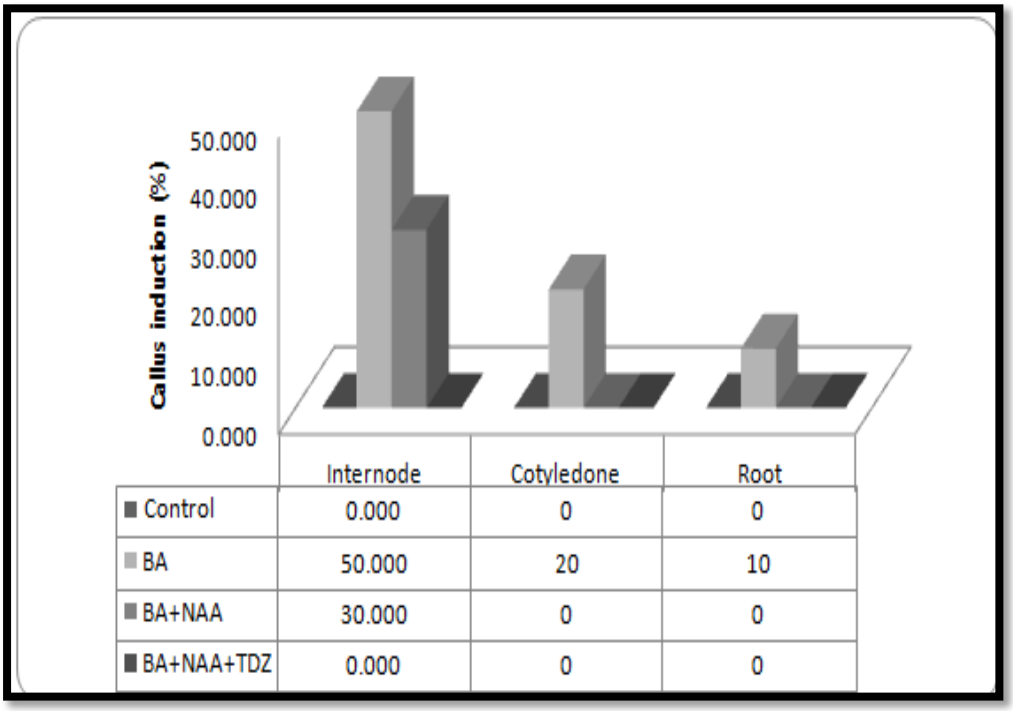


Figure 1-Effects of NaOCl solutions at different concentrations and time periods.

Figure 2-Seedlings of *P. vulgaris* L.seeds after 7-14 days.

Effects of BA and NAA on callus induction from bean explants

The explants of *P. vulgaris* L. showed callus induction after seven days of incubation and well-developed callus was obtained after 21 days. The explants showed a swelling (Figure-4B) soon after a week of incubation, followed by the emergence of callus all over the explants. The swelling of the explants observed in *P. vulgaris* may be due to the internal pressure developed during the incubation. Similar to this result, the swelling of explants during the induction of callus was reported in the hypocotyl and leaf explants of *Meconopsis simpficifofia*, nodal explants of *Dendrocalamus hamiltonii* Nees, and leaf explants of *Mentha piperita* (L.) [18]. Benzyl adenine (BA), as a plant growth regulator with 5 mg/L, was observed to be compatible for the high frequency of callus induction from stem explants compared with cotyledons and roots with values of 50, 20, and 10 %, respectively (Figures-3,4). When the stem internode explants were cultured in MS medium with 4.5 mg/l of BA and 0.5 mg/l NAA, the highest percentage of callus was recorded, in comparison with cotyledons and root explants. It has been proved that adenine and adenosine have cytokinin-like activities and help growth amelioration when provided to the culture medium [19]. In the present study, the stem explants of *P. vulgaris* responded well when cultured on a medium provided with combinations of auxins and cytokinins. This suggests that the callus induction and shoot regeneration from the selected explants ensured the genotype and mixtures of growth regulators as reported by Zhang *et al.* [20]. The callus obtained was also maintained on appropriate culture conditions, either to regenerate the plantlets or to provide pharmaceutically bioactive compounds**.**



	Internode	Cotyledone	Root
Control	0.000	0	0
BA	50.000	20	10
BA+NAA	30.000	0	0
BA+NAA+TDZ	0.000	0	0

Figure 3-The interaction between plant growth regulator and explant types on the callus induction in bean pinto**.**

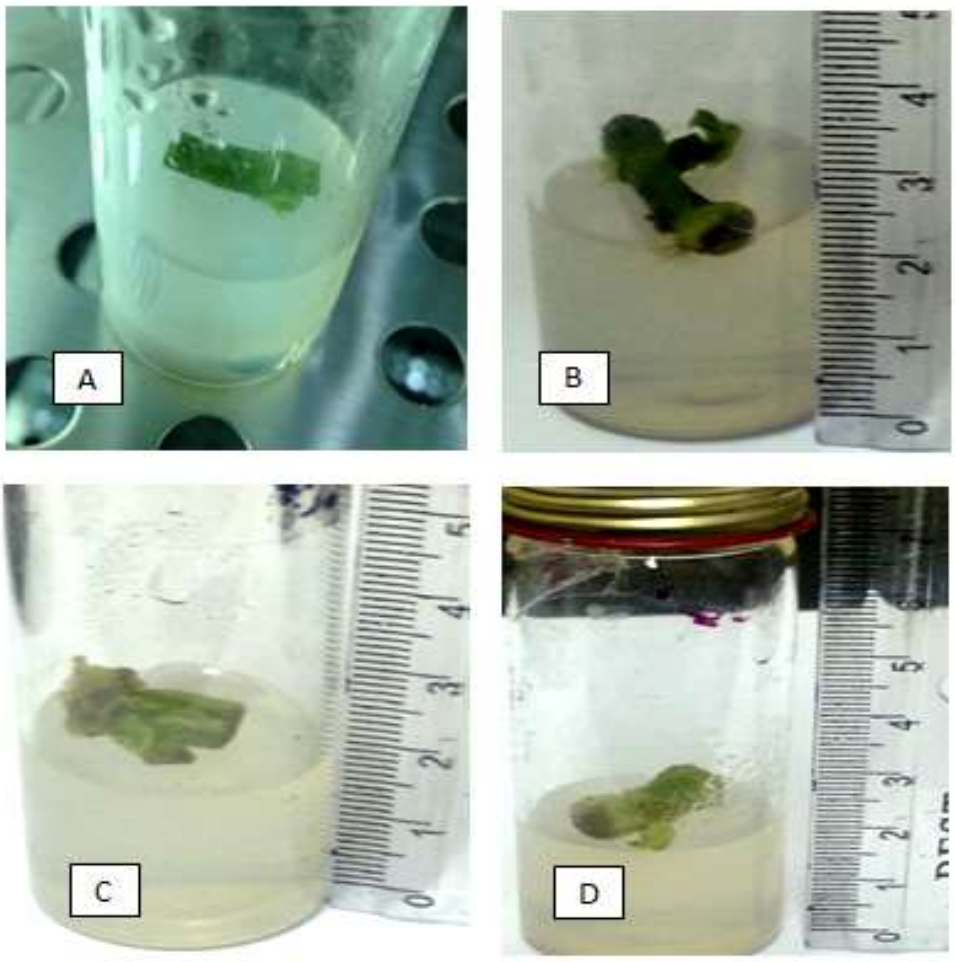


Figure 4-A: Stem explants; B; Swelling explants showing the induction of callus; C: Callus initiation on medium provided with BA; D: Callus initiation on medium provided with NAA.

Effects of TDZ with BA and NAA on shoot organogenesis

The optimum results were obtained by growing internodes (stem) on MS medium with 4.5 mg/l BA and either 0.5 mg/l NAA or 0.1 mg/l TDZ- derived shoots (Figures- 5 and 6). Rossetti [21] also reported the need for different hormone combinations for different developmental stages. A comparative study between different methods of shoot and plantlet regeneration in red kidney bean was accomplished by the use of different combinations of BAP and NAA [22]. Different publications were concerning the crucial role of BA, TDZ and NAA on *in vitro* determination of bean embryogenesis and organogenesis. Medium composition of shoot initiation is important in the process of regeneration through organogenesis followed by a development of higher number of buds and shoots in this medium [18]. In the present study, BAP and TDZ played a substantial role in the organogenic structures formation of pinto bean cultivars. Cytokinins stimulate the division of plant cell and contribute in the cell cycle control [23]. The current study is in accordance with that of Malik and Saxena [24] who recorded that a culture medium containing 5 µM BAP was the optimum for shoot initiation from *P. vulgaris* leaf explants, in comparison with the culture on MS medium devoid of BAP. Another study demonstrated that the combination of BAP and TDZ at 5 µM was the favorable for shoot initiation of *P. angularis* cvs. [25].

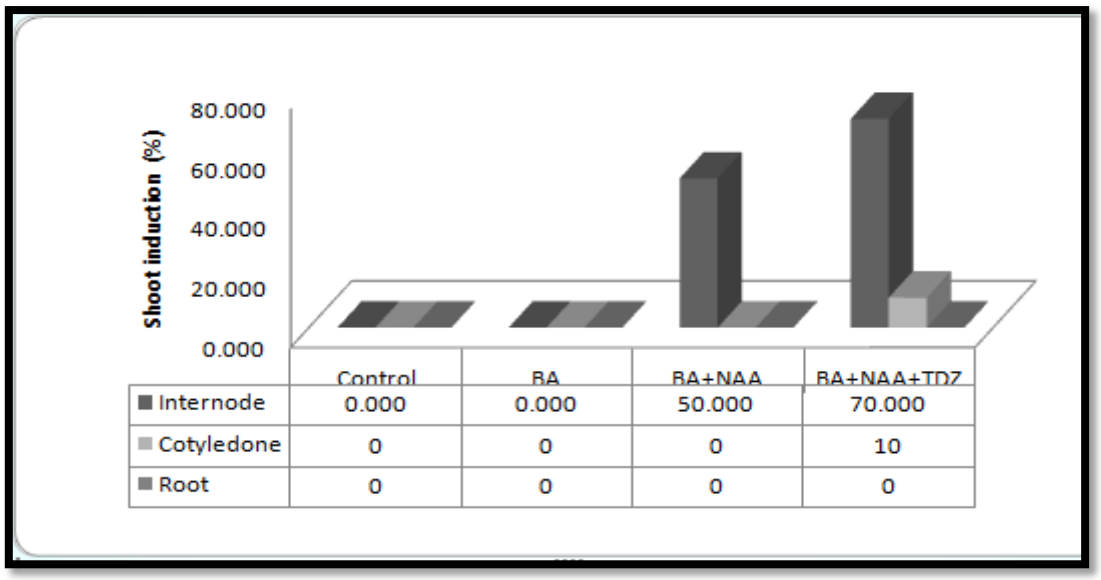


Figure 5-Effects of the combination of TDZ with BA and NAA on shoot organogenesis.

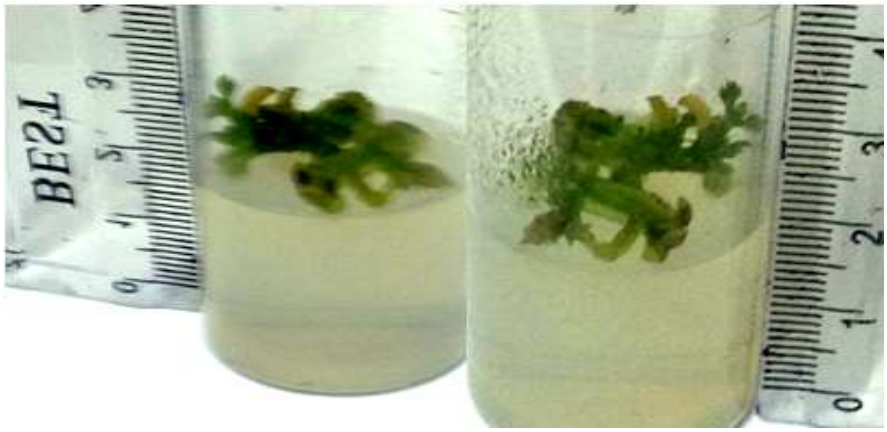


Figure 6-Establishment of shoots on medium provided with BA, NAA and TDZ.

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