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INCREASING THE PRODUCTIVITY OF SEEDLINGS AND TOTAL YIELD OF HUSK TOMATO PLANTS BY USING TISSUE CULTURE TECHNIQUE

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ABSTRACT

A relibale protocol for in vitro propagation of husk tomato *(Physalis ixocarpa, L.) plant var. Americana* was established. Shoot tips explants were used and different cytokinins were compared. Shoot proliferation was obtained from shoot tips at relatively height frequency when cultured on Murashige & Skoog (MS) medium supplemented with high level of cytokinins (4.0 mg/L), while it had a great inhibitory effect on root initiation. Benzyladenine (BA) surpassed significantly than Kinitin (Kn) in stimulating of the lateral bud-break and increasing shoot branching in the plants. Seedlings derived from tissue culture produced plantlets which were shorter, thicker, with more leaf chlorophyll content, less leaf temperature, transpiration, bigger leaf area and gave higher total yield than those derived from seed sowing

INTRODUCTION

Physalis plant is a solanaceous crop produced small yellow fruit at maturity stage. The fruits are normally covered with the calyx during all stages of fruit development. The plants tolerated to salinity up to 3000 ppm (Mahmoud, 1995). The importance of tolerance to salinity in the arid lands is very important. However, the plants produce exotic fruits, which are popular locally abroad. The fruits also are rich in vitamins, minerals, proteins, carotene, sugar and organic acid (Tindall, 1983).

Several laboratories have been reported the ability to regenerate direct shoot from leaf section (Zorzoli *et al.*, 1990; Lui and Li 1988 and Stommel and Sinsen 1991, on tomato). Within the availability of the previous micro propagation, greater number of meristemic nodules and buds per leaf were induced (Roland and Elizabeth, 1992). Plant regeneration is also possible from primary and sub cultured callus induced from immature leaves (Nehara et al ,.1988) but shoots regeneration from callus is not only slow but it also induced somaclonal variation which is undesirable in propagation , germoplasm conservation and production of transgenic plants (Nehara *et al* ,1988). Growth regulators namely auxins, cytokinins, gibberellins and abscisic acid are important in tissue culture. The differentiation and organogenesis of tissue become feasible only on the addition of one or more of these classes of hormones to the culture medium. The ratio of hormones required for root or shoot induction varies considerably with the tissue which seem directly correlated to the quantum of hormones synthesized at endogenous levels within the cells of the explant (Razdan 1994). Cytokinins play an important roll in the number and length of shoots production Increasing the concentrations of cytokinins caused an increase in shoot number per explant

but the obtained shoots are short.Benzyladenine (BA) and Kinetin (Kn) have been demonstrated in shoot proliferation *in-vitro*. These compounds appear to have strong Cytokinins like effects on a wide range of species (Razdan 1994).

Plants grown *in-vitro* usually do not possess productive mechanism against desiccation, characterized with reduction in epicuticular waxes, impaired stomata function and a depression in photosynthetic competence (Preese,1992).Moreover, plantlet leaf anatomy and physiology are affected *in-vitro*. Up till now very limited information are available to answer the question of why tissue culture plants are more superior than conventional plants in growth and productive potentials. This may be contributed to the use of the true potential of tissue culture plants beside the appropriate management for their specific requirements (Robinson *et al.*, 1993). However, the main visual differences between the two planting materials are in their morphology and growth habits during their development (Drew and Smith, 1990). More observations on tissue culture plants, for example in strawberry, it tended to be more bigger in vegetative growth, higher in rate of photosynthetic and dry matter assimilation beside efficient physiologically leaf area and vigorous roots than conventional plants (Eckstein and Robinson, 1993).

The aim of this work is consists of two parts, the first: obtained mass production of direct shoots regenerated from leaf sections and study their growth habits in comparison with those of normal propagation (Seed sowing). The second: Comparison between tissue culture and seed sowing in total yield, number and fruit fresh weight

MATERIALS AND METHODS

This work was carried out during the period from 2001 and 2002 seasons in the Arid Land Horticulture Research Unit (ALARU), faculty of Agriculture, Ain Shams University. Our experiment consists of two parts Firstly: Direct shoot regeneration from leaf section.
Secondly: Comparison between tissue culture and seed sowing.

Firs experiment: Direct shoot regeneration from leaf section.

Hybrids seeds of physalis were sown on December 25th 2001 in the propagation trays containing a mixture of peat moss and sand at ratio of 1:1 (v:/v). The process of germination was done under controlled greenhouse conditions at 26°C and 80% relative humidity. After 15 days from germination, Hybrids seedlings of the physalis were removed from the trays and transferred to the tissue culture laboratory where the youngest expanded leaves were removed and used as explants. After that the explants washed several times with tap water and surface sterilized inside the Laminar Flow Hood, by immersing in 1% sodium hybpclorite for 15 min then rinsed three times with sterilized distilled water. The leaflets were unfolded by cutting both the mid vein and then the blade horizontally at the base. The leaf pieces thus separated were placed on the surface of the media. The basal nutrient media

used contained macro and microelements according to Murashige and Skoog (MS) medium (1962). The pH of the media was adjusted at 5.7+ 0.1 before the addition of agar. The media were distributed into culture tubes where each tube contained 15 cm and sterilized by autoclaving at 121° C for 15 min.

Treatments: A- Effect of Cytokinins: Benzyladenine (BA) and Kinitin (Kn) were added at concentrations ranged from 0.0 (control), 1.0, 2.0 and 4.0 mg/L. were used.

Each treatment consisted of three replicates and 10 tubes resembled each replicate. The culture tubes were kept at constant temperature for 26+ 2°C and sufficient fluorescent light of 1500 Lux for 16 hours photoperiod. Data were recorded after 4 weeks from culture and were subjected to proper statistical analysis of variance according to Snedecor and Cochran (1980).

Second :Transplanting of plantlets from *in-vitro* to the plastic house.

A-Vegetative stage:

All shoots obtained from the previous experiment were transferred to the rooting medium containing IBA at 1.0 mg/L. After two weeks, the complete plantlets produced were transferred from the culture tubes into plastic pots filled with peat-moss and sand at a ratio of 1:1 (v:v) and immediately transferred to controlled plastic-house under mist and fertilized with Hoglands solution once a week for a period of 14 days.

Data were recorded after 3 weeks from culture and were subjected to proper statistical analysis of variance according to Snedecor and Cochran,(1980).

B: Total yield

Both the seedlings produced by tissue culture and by seed sowing were transplanted into plastic house in Shalakan Experiment Station,Faculty of Agric.Ain Shams Univ. After that the complete plants were transplanted into the open field of Barrage Horticultural Experimental Station, which belong to the Agriculture Research Center on March 15th 2001. The treatments were arranged in 3 replicates using a randomized complete blocks design. The size of each replicate was 13.5 m^2 and consisted of 3 rows. Each row was 4.5 m long and one meter wide . The distances between plants was 80 cm. All agriculture practices were done. The fruit were picked twice a week and the data about total yield were subjected to statistical analysis using the analysis of variance methods according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

First Experiment: Direct shoot regeneration from leaf section.

The morphogenetic response of leaf section of physalis to various concentrations of cytokinins is show that both cytokinins treatments gave rise to the maximum percentage 100% of direct shoots but at the same time suppressed the development of any root proliferation. On the other hand the untreated explant (control) failed to regenerate and died after few days.

These results were agreement with many previous results of Behli and Lesley, 1976 on tomato,Deveikat and Lyrene. 1988 on bluberry and Narender and Cecil 1989 on strawberry who suggested that cytokinins have a great inhibitor effect on root initiation and the only success was for the development of shoots.

Concerning the parameter of shoot number per explant data in Fig (1) reflected that this character depended to a great extent on both the applied concentrations and type of cytokinins used, however, increasing the levels of both cytokinins were sufficient to enhance the capability of explants to produce more shoots especially with the highest level 4.0 mg/L which produced the highest number of shoots. Some previous researchers supported our present experiment that there was direct relationship between the higher concentrations of cytokinins and the increase in shoots number. However, presence of cytokinins in the media depressed the apical dominance and consequently activated the axillary's buds, which increase the opportunity to proliferate. Thus, raising the concentrations of cytokinins increase the bud proliferation and the formation of multi apexes plantlets (Anderson, 1993) on strawberry; (El-Zeiny, 1997) on tomato. On the other hand when we put the two cytokinins in comparison data cleared that BA surpassed significantly than Kn in shoot production Fig (1). These results coincided with (El-Zeiny), 1997 on tomato.

Regarding the parameter of shoot length, Fig (2) suggest that the addition of all applied cytokinins from 1.0 to 4.0 mg/L led to a reduction in shoot length than the control treatment. So, the shortest shoots resulted from the highest level 4.0 mg/L in both cytokinins under treatment. On the other side (Kn) treatments produced the longest shoots than the BA application. The trend of these results was in agreement with some previous work (Anderson.1993). On strawberry and (El-Zeiny, 1997 and 2002) on tomato and pepper respectively.

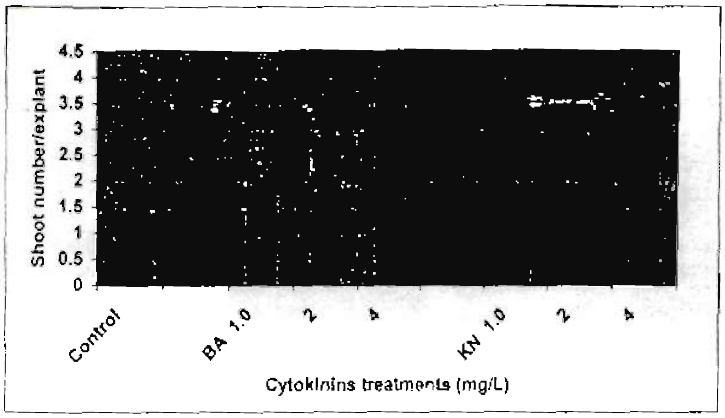
The response of callus formations as affect to the various cytokinins data in Fig (3) showed that no callus was formed in all levels of cytokinins treated explants except the highest level 4.0 mg/L in the two cytokinins under this investigation. (BA) produced the heaviest callus weight as compared with Kn. Similar results were obtained by El-Zeiny 2002 on pepper.

Concerning the shoot fresh weight, data in Fig (4) cleared that addition the examined cytokinins from 1.0 to 2.0 mg/L increased the weight while the high level 4.0 mg/L led to a reduction in this character. Because of the previous treatment of cytokinins at 4.0 mg/L produced callus growth at the base of shoots which was undesirable character as it utilize nutrient from the media, and slow down nutrient uptake by shoot growth causing less in shoot weight (Barghchi and Alderson, 1993 and El-Zeiny 2002).

Second experiment: Transplanting of plantlets from *in-vitro* to the plastic house.

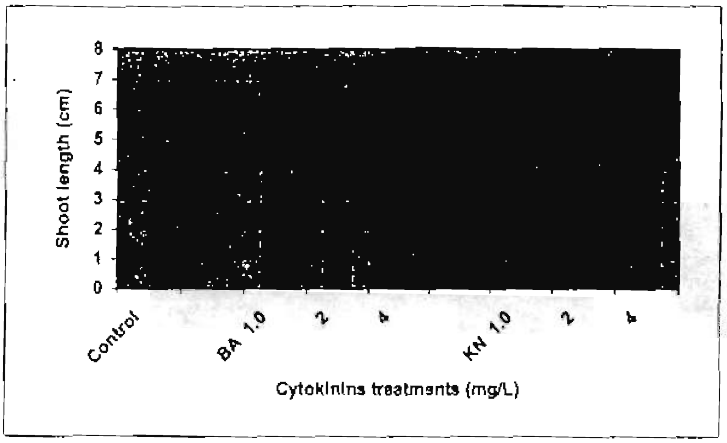
A-Vegetative growth

Data in Fig (5) illustrated that seedling from tissue culture produced number of shoots more than the seed sowing plant. These results may be due to the high levels of cytokinins used for shoot proliferation in tissue



L.S.D at 0.05
Treatments : 0.06
Concentration: 0.08

Fig (1): Effect of cytokinins treatments on shoot number of physalis developed from leaf section.



L.S.D at 0.05
Treatments : 0 42
Concentration: 0.73

Fig (2): Effect of cytokinins treatments on shoot length of physalis developed from leaf section.

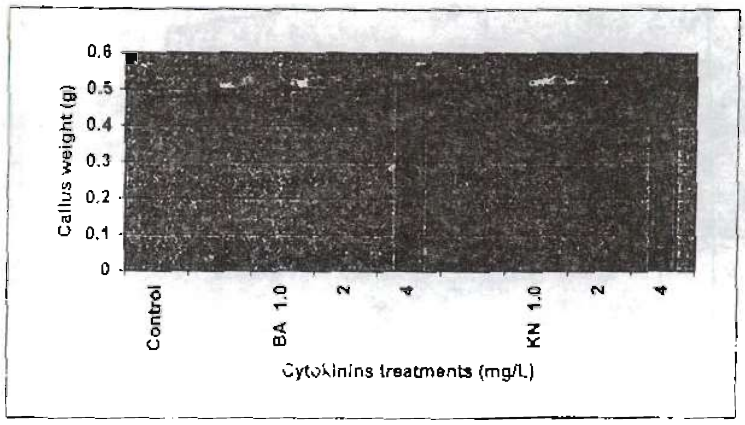
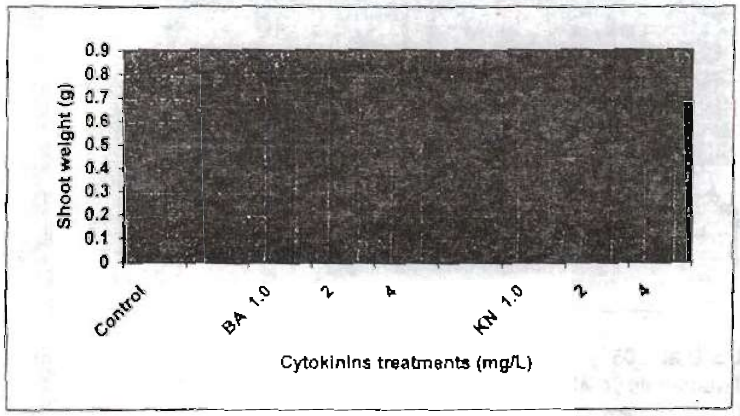


Fig (3). Effect of cytokinins treatments on callus weight of physalis developed from leaf section.



L.S.D at 0.05
Treatments : 0.06
Concentration: 0.04

Fig (4): Effect of cytokinins treatments on shoot weight of physalis developed from leaf section.

culture environments, which stimulated the lateral bud brake in the micro shoots and consequently increased branching. (El-Zeiny 2002) on pepper

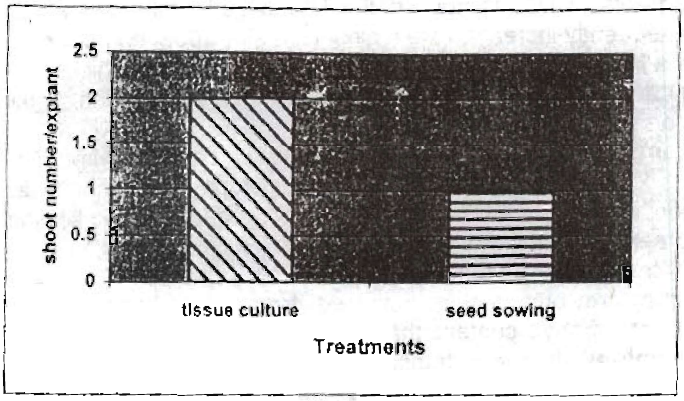
The data in Fig (6) cleared that tissue culture plants were thickness in stem diameter compared with that produced by common propagation. Similar results were done by (El-Zeiny 1997) on tomato.

Histograms in Fig (7A,7B) Showed that tissue culture environments produced plants characterized with more leaf chlorophyll content and less leaf transpiration as compared with the normal propagation (Seed sowing) Our results agreement with that data obtained with (Robinson et al,1993) on banana and (El-Zeiny 1997) on tomato.

From the previous results obtained from plants tissue culture possessed high chlorophyll content that induce more photosynthetic rates beside a comparatively low leaf transpiration, this in turn build high yield of carbohydrates beside the increase in water potential due to the low leaf transpiration. These factors gave rise to more cell division and enlargement inducing more vigorous tissue culture plants than those of conventional plant (Robinson *et al.*, 1993 and El-Zeiny 1997).

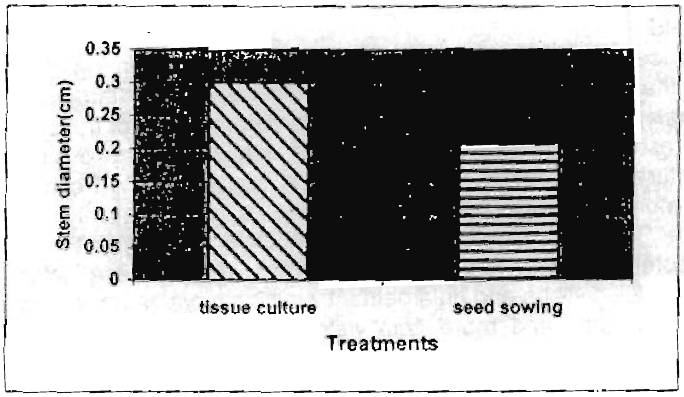
B-Total yield

Concerning the parameter of yield per plant, data in Fig (8A,8B,8C) exhibited that the tissue culture plants were significantly superior to the conventional plants in producing higher total yield, number of fruits per plant and fruit fresh weight. These data may be due to the previous results of tissue culture plants which possessed high leaf chlorophyll content that induced more photosynthetic rates beside a comparatively low leaf temperature. This in turn built high yield of carbohydrates beside the increase in water potential due to comparatively low transpiration. These factors gave rise to more cell division and enlargement inducing more vegetative vigorous tissue culture plants and more total yield than those of conventional plants (Robinson *et al.*, 1993).



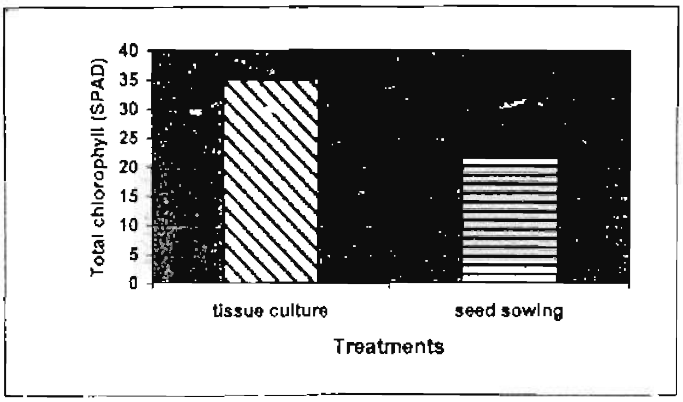
L.S.D at 0.05:
Treatments: 0.13

Fig (5): Comparison between tissue culture and seed sowing in shoot number

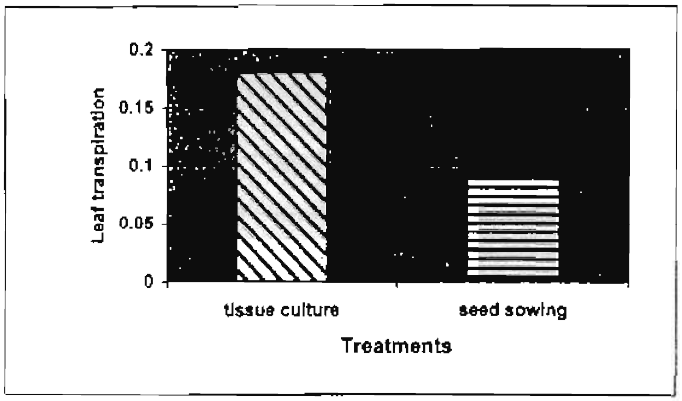


L.S D at 0.05:
Treatments: 0.16

Fig (6): Comparison between tissue culture and seed sowing in stem diameter

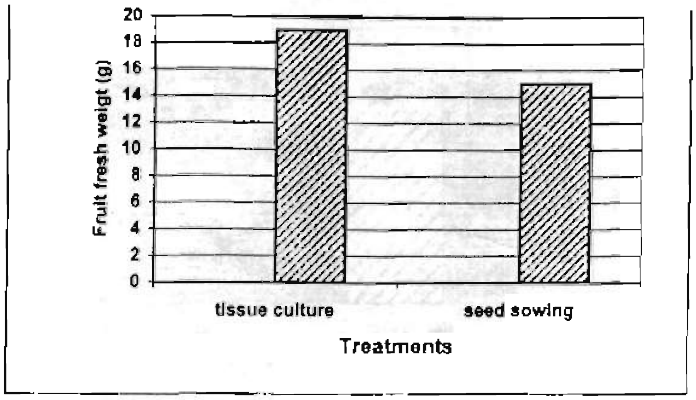


L.S.D at 0.05:
Treatments: 2.26

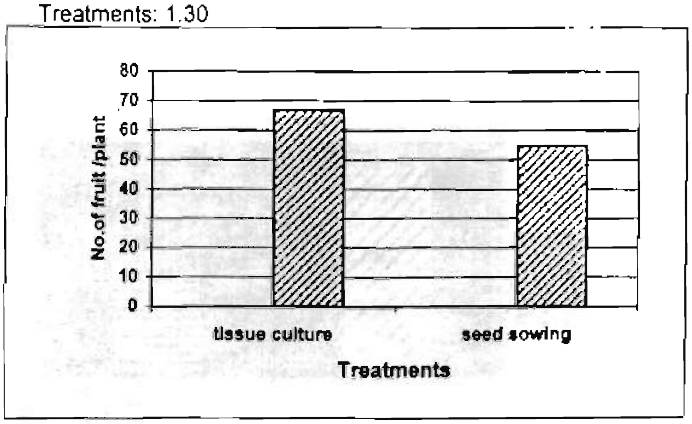


L.S.D at 0.05:
Treatments: 0.1

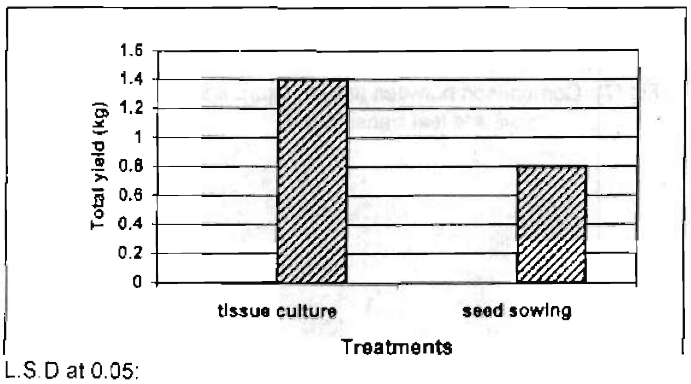
Fig (7): Comparison between tissue culture and seed sowing in total chlorophyll and leaf transpirtation



L.S.D at 0.05
Treatments: 1.30



L.S.D at 0.05:
Treatments: 2.26



L.S.D at 0.05:
Treatments: 0.26

Fig (8): Comparison between tissue culture and seed sowing in total yield,number of fruits per plant and fruit fresh weight

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زيادة إنتاجية الشتلات والمحصول الكلي لنبات الحرنكش باستخدام تكنيك زراعة الأنسجة

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وضعت الأسس الثابتة لإكثار نبات الحرنكش حيث استخدمت القمة النامية مع السيتوكينينات المختلفة للمقارنة . ودلت النتائج على أن عند زراعة القمة النامية في بيئة موراشيج واسكوج (M*S) المحتوية على التركيز العالي من السيتوكينينات (٤ مليجرام / لتر)أدت الى زيادة عدد الأفرع المتحصل عليها بنسبة عالية وسريعا ولكن في نفس الوقت كانت تلك المعاملة مثبطة لتكشف الجذور. كذلك دلت النتائج على أن البنزايل أدينين كان أكثر السيتوكينينات تفوقا معنويا مقارنة بالكينيتين كما وجد أن الشتلات الناتجة من زراعة الأنسجة كانت قصيرة وسميكة وأكثر تفريعا ، وكانت أوراقها ذات محتوى عالي من الكلوروفيل وذات مساحة كبيرة ودرجة حرارتها و معدل نتحها أقل من تلك المتحصل عليها من زراعة البذرة العادية. كذلك كان المحصول الكلى لكل نبات ناتج من زراعة الأنسجة أكثر انتاجية مقارنة بتلك الناتج من الزراعة التقليدية (انتاج الشتلات بالبذرة).