
I. ALİ NUMAN KIRAÇ
TARIM KONGRESİ VE FUARI
27-30 Nisan 2011
ESKİŞEHİR
BİLDİRİLER/
PROCEEDINGS
CİLT I / VOLUME I
1st National Agriculture Congress and
Exposition on behalf of
Ali Numan Kıraç with
International Participation
April 27-30, 2011

ALİ NUMAN KIRAÇ

Ali Numan Bey 1897'de Bursa'da doğmuş, Afyon İdadisi ve Bursa Ziraat Mektebi'nden mezun olmuştur.

Bursa Ziraat Mektebi'nde öğretmen olarak başlayan ziraatçılık serüveni, Ankara'da Gazi Çiftliğinde devam etmiş ve eğitimini geliştirmek için Atatürk tarafından Amerika Birleşik Devletlerine gönderilen ilk ziraatçı olmuştur. 1927-1931 yılları arasında Kansas Ziraat Koleji sonra Nebraska Üniversitesi'nden mezun olarak Türkiye'de dönmüş ve çalışmalarına Eskişehir'de devam etmiştir. "Kıraç" soyadı kendisine Atatürk tarafından verilmiştir.

Ali Numan Bey ve eşi Semiha Hanım on yıl sürece oğulları Can ve İnan'la Eskişehir'in beş kilometre dışında Karacaşehir eteklerinde kurudan Drayfarming Deneme İstasyonu'nda yaşamışlardır.

Ali Numan Kıraç, Türk tarımının gelişmesi için eleman yetiştirme, yeni metotlar ve ürünler geliştirme çalışmalarını 31 yıl sürdürmüştür. Türk tarımında kullanılan pek çok yeni tür tohumu geliştirmiştir. Tarım Bakanlığı Müsteşarlığı ve Devlet Üretme Çiftlikleri Genel Müdürlüğü'nden emekli olarak İstanbul'a taşınmış ve 30 Haziran 1954 günü hayata veda etmiştir.

Uluslararası Katılımlı
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MORPHOGENESIS OF SOME AGRICULTURE SPECIES AT *IN VITRO* CONDITIONS

Liljana KOLEVA GUDEVA[1]

ABSTRACT

At the beginning of the XXI century, the perspectives of the plant biochemistry and physiology are directed to examine the capability of plant cells and tissue culture for vegetative propagation. The method of *in vitro* cultivation of plant cell and tissue cultures is use for vegetative propagation (micropropagation) of plants. The vegetative propagation of the plants in *in vitro* conditions enables to abbreviate the process of selection, enhance the genetic stability of plants and improve the production of healthy plants free of viruses.

In this paper the results from the experimental work from the capacity of *in vitro* morphogenesis and micropropagation of some agricultural species are presented (*Capsicum annuum* L., *Lycopersicon esculentum* Mill., *Cucumis sativus* L.), The results were obtains form different initial explants cultivated *in vitro* on different hormonal medias, and were done at the Laboratory of biotechnology at the Department of plant biotechnology, genetic and selection, Goce Delcev University – Stip, R. of Macedonia.

Keywords: vegetative propagation, phytohormones, regeneration, morphogenesis.

INTRODUCTION

The production and maintaining of plant tissue culture is in mass use today, because with this procedure in short time and at small space can be obtained possibly unlimited number of genetically identical plants. With this method, not only that the possibility of getting free of viruses plant material and better genetic stability of the regenerated plants is real, but it improves the morphological and biological characteristics of the culture as well (Koleva Gudeva, et al., 2001).

Morphogenesis in plants is a complex phenomenon and is being regulated by numerous factors and in-between relationships of plant organs, tissues and cells also. The correlation between cells tissues and organs of a plant plays important role in the growth and development in *in vivo* and in *in vitro* conditions. The study of such complex system can be simplified with cell, tissue or organ isolation and their cultivation *in vitro*. In such conditions, the influence of certain factors on the organogenesis and differentiation of the plant tissue can be traced, most of which, examined the effect of plant hormones as an important factor in the plant morphogenesis. Today, vegetative propagation *in vitro* conditions, finds wide application in horticulture, gardening, orchards, winegrowing and forestry.

MATHERIAL AND METHODS OF WORK

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The main objective of this research was to set up a culture of meristem and nonmeristem explants, to get to know the properties of the tissues *in vitro*, and to see the possibilities for their morphogenesis and micropropagation.

Isolation of initial explants

As an initial material to work from the meristem explants, apical buds with size up to 3 mm, and meristem form the apical buds with size up to 0.5 mm, were used. From the non meristematic explants were used whole cotyledones or a part from cotyledons, hypocotyls, nods and internodes. Pepper anthers were used as initial explants for androgenesis and obtaining of haploid regenerants. All initial explants isolated from seeds or from plant material *in vivo* (pepper anthers) was previously subject to sterilization.

Sterilization of plant material

The pepper, cucumber and tomato seeds were sterilized on several steps. First, the seeds were rinsing with tap water, and then were left immersed in distilled water for a few hours. Then, the seeds are left for 15 seconds in 70% C_2H_5OH, following 10 minutes in 5% $Ca(ClO)_2$, 10 minutes 1% Isosan-G, and at the end is rinsed a few times in sterile water and after that are being put on ½ MS (Murashige and Skoog, 1962) mineral solution.

The plant material from which the initial explants were isolated, like flower buds from which anthers are isolated for example, were sterilized in the following manner: rinsing with tap water, than in distilled water, followed by 15-20 seconds in 70% C_2H_5OH, Tween 20 enriched, 10-15 minutes in 5% $Ca(ClO)_2$ enriched with Tween 80, and on the end rinsing of the explants with sterile water for a few times. Sterilized this way, the initial explants were cultivated on MS media in which various concentrations and combinations of plant hormones were added.

Plant growth medium ingredients

Almost all of the plant species were cultivated on MS mineral solution containing 3% sucrose, 0,7% agar, 100 $mg \cdot l^{-1}$ inositol, 200 $mg \cdot l^{-1}$ casein hydrolysate, 0,1 $mg \cdot l^{-1}$ B1, 1,0 $mg \cdot l^{-1}$ B6 and 0,5 $mg \cdot l^{-1}$ nicotinic acid. Different phytohormones were used in a medium such as: IAA (indole-3-acetic acid), IBA (indole-3-butyric acid), NAA (α-naphtaleneacetic acid), BAP (6-benzylaminopurine), BA (N6-benzyladenine), KIN kinetin (6-furfuryl aminopurine), ZEA zeatin (N^6-4 hydroxyl-3-methyl -trans -2butenyl anunopurine), 2iP (N^6-2-isopentyl adenine) and 2,4 D (2,4-dichlorophenoxy acetic acid).

The androgenetic potential of the pepper was examinee on medium developer from Dumas de Valux, et al., 1981 method for pepper anther culture.

Growth conditions

The seeds were put to germination on basal media, all of the explants as every next cultivation on new plant hormone media were cultivated in climate chamber with controlled conditions and temperature of 25±2ºC, photoperiodic of 16/8 light/dark, 50% relative humidity, and 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity.

The growth conditions for examination of morphogenetic potential in pepper anther culture and the ability for somatic embryogenesis was also set up according the Dumas de Valux, et al., 1981 method.

RESULTS AND DISCUSSION

Strumica region is mainly oriented towards agriculture, so our interest in the Faculty of Agriculture, Goce Delcev University – Stip, is mainly oriented to *in vitro* cultures of vegetable species. In the

conventional production, leading position have the tomatoes, peppers and cucumbers, so the emphasis in the research on regenerative ability in *in vitro* conditions is given to these leading crops in the region and in worldwide scale.

Micropropagation and of pepper *Capsicum annuum* L.

In the research of the regenerative potential of pepper in *in vitro* culture, from the meristem tissues apical buds and isolated meriste were used as a initial explants. From the non meristematic tissues cotyledons and hypocotyls segments were used such as initial explants for further morphogenesis in tissue culture. Successful regeneration of pepper from apical buds were obtained and adapted in non-sterile conditions (Figure 1). From the nonmeristem explants the morphogenesis takes place towards callus formation and very rarely results in formation of leaf rosettes. Leaf rosettes obtained form nonmeristematic tissue turn their further organogenesis not to form shoots just to proliferate in the medium without plantlet formation. (Table1).

Androgenesis of pepper *Capsicum annuum* L.

The androgenetic potential of the pepper was also researched and the ability for embryos induction in pepper anther culture was examined too. Studies were performed on different media with different hormonal combinations and concentrations and to stimulate the androgenetic ability, several different incubation temperature treatments were used. From all the tested media and treatments haploid embryo production is accomplished only with the method of Dumas de Valux, et al., 1981 (Table 1, Figure 2).

Tests were performed on 21 different genotypes of pepper and seeds from 4 genotypes were collected (Kurtovska kapija, Golden medal. Feherozen and Piran), which is the subject of further cytogenetic and other research on the molecular level. From regenerants of these four genotypes were produced several selection lines involved in the process of selection of peppers.

Micropropagation of tomato *Lycopersicon esculentum* Mill.

As experimental material for tomato micropropagation, apical buds, cotyledons and hypocotyls were used. The effect of different concentrations of the cytokinins BAP and KIN, in combination with different concentrations of the auxins IAA and IBA in the media was observed. BAP + IBA combination has proved the most effective leaf rosettes formation from and apical buds in comparison to other initial explants showed the greatest potential for creating plantlets and their multiplication in *in vitro* conditions (Table 1, Figure 3).

Micropropagation of cucumber *Cucumis sativus* L.

Apical buds, cotyledons and hypocotyls segments were used as initial explants for micropropagation of cucumber in *in vitro* conditions. As with other species, also and with the cucumbers, predictably maristematic tissues had greater potential for creating shoots in culture from the nonmeristematic tissues (Fig. 4). More hormonal combinations in MS medium were examined and the ones on which greatest effect was observed and those combinations are shown in the Table 1.

Table 1. Revision of some vegetable species micropropagated in *in vitro* conditions.

Species	Explant	Medium+Growth Regulators $mg \cdot l^{-1}$	Results
Capsicum annuum L.	apical buds	MS + 5.0 BAP + 0.5 NAA MS + 10.0 BAP + 0.5 IAA MS +1.0ZEA	callus shoots
	anthers	CP + 0,01 KIN + 0,01 2,4D R_1 + 0,01 KIN	embryos

	hypocotyls 1/3 cotyledons	MS + 10.0 BAP + 0.5 NAA MS + 30.0 BAP + 1.0 IAA MS + 5.0 ZEA MS + 2.5 2iP	callus
Lycopersicon esculentum Mill.	apical buds	MS + 4.5 BAP + 0.3 IBA MS + 6.0 BAP + 0.4 IBAA MS + 4.5 KIN + 0.3 IAA	shoots
	hypocotyls 1/3 cotyledons	MS + 1.5 BAP + 0.1 IBA MS + 3.0 KIN + 0.1 IAA MS + 6.0 BAP + 0.4 IBA	callus
Cucumis sativus L.	apical buds	MS + 11.0 KIN + 3.5 IBA	shoots
	hypocotyls	MS + 2.0 KIN	callus
	1/3 cotyledons	MS + 6.5 BA+10.0 2,4 D	callus

CONCLUSION

The application of *in vitro* techniques for mass micropropagation of plants have great success with the ornamental, fruit, forest, horticultural and medical species. In *in vitro* conditions full recovery of more than 300 plant species is achieved, and the method has a special significance in the research of several fields as plant physiology, biochemistry, biotechnology, molecular biology and others. Today, without the use of *in vitro* methodology , many sophisticated and complex processes at the molecular level can not be imagined and implemented, which challenge the XXI century.

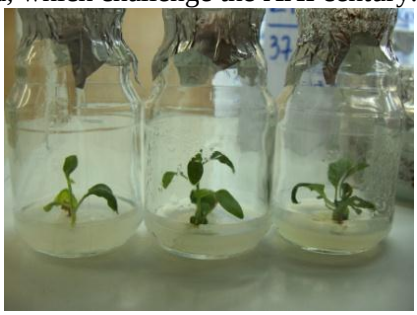

Figure 1. Shoot culture of pepper *Capsicum annuum* L.

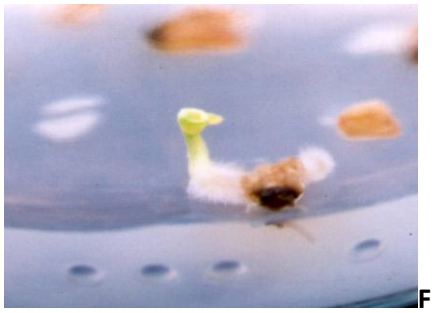

Figure 2. Development of somatic embryo occurred on the pepper anther.

Figure 3. Shoot culture of tomato *Lycopersicon esculentum* Mill.

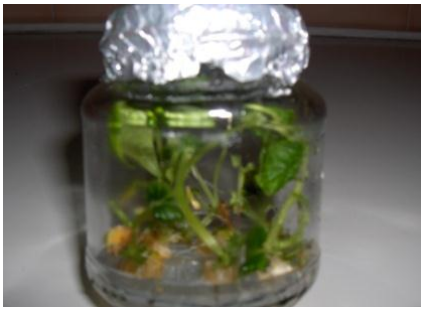

Figure 4. Shoot culture of cucumber *Cucumis sativus* L.

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