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PREFACE

Dear Colleagues,

In your hands are the *Proceedings* of the 7th International Scientific Agricultural Symposium "AGROSYM 2016", which I hope you will find useful in your research, education and professional activities and endeavors.

AGROSYM 2016 themes cover all branches of agriculture and are divided into seven sessions: 1) Plant production, 2) Plant protection and food safety, 3) Organic agriculture, 4) Environmental protection and natural resources management, 5) Animal husbandry, 6) Rural development and agro-economy, and 7) Forestry and agroforestry.

Keynote papers dealt with organic agriculture from different perspectives as well as international cooperation in agricultural and rural development, climate change and forestry. Organic farming is a holistic production management system of agricultural, food and other products, combining good agricultural practices, a high degree of biodiversity, conservation of natural resources, and application of high standards of animal welfare.

Food production, food security and production of raw materials for other industries determine multiple environmental, economic and social roles of agriculture (including crop production, animal husbandry, forestry and fisheries) in all countries, both developed and developing ones, as well as its multi-functionality; including its contribution to rural development. This is clearly highlighted in the numerous contributions included in these *Proceedings* alongside the challenges that agriculture sector is facing and the negative impacts of industrial agriculture on the environment.

I do believe that the research results reported here will be significant in the dissemination of knowledge to the wider audience about the importance of agriculture as a strategic sector in many countries.

AGROSYM 2016 made an important contribution to the agriculture theory, knowledge and practice in different areas. During the four-day symposium on October 6-9, 2016, over 1100 contributions were presented (either as oral or poster presentations) to more than 1000 participants representing 78 countries.

The *Proceedings* comprise an edited selection of the papers submitted to the symposium. Each paper included in the *Proceedings* was positively reviewed by referees.

AGROSYM 2016 was made possible through the commitment and contributions of a wide range of partners and sponsors. Many thanks to all authors, reviewers, session moderators and colleagues for their help in editing these *Proceedings*. Special thanks go to all co-organizers for their unselfish collaboration and comprehensive support.

East Sarajevo, 05 October 2016

Prof. Dušan Kovačević, Faculty of Agriculture, University of Belgrade

Proceedings Editor in Chief – President of the Scientific committee of AGROSYM 2016



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IN VITRO SHOOT AND ROOT REGENERATION IN ERCIS CABBAGE (BRASSICA OLERACEAE VAR. CAPITATA)

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Abstract

Ercis head cabbage which is a population local to the Van province of Turkey was tested for ability to regenerate shoots and roots *in vitro*. Cotyledon and hypocotyl explants were excised from 7 day old *in vitro* seedlings, germinated on Murashige and Skoog (MS) medium, and cultured on MS medium supplemented with benzylaminopurine (BAP) (0.5 mg/l, 1.0 mg/l, and 2.0 mg/l) and naphthaleneacetic acid (NAA) (0, 0.05 mg/l, and 0.1 mg/l). The highest shoot regeneration (100%) was achieved in hypocotyls explants with MS7 (MS + 2 mg/l BAP) and 91.6% with MS6 (MS + 1 mg/l BAP + 0.1 mg/l NAA), MS4 (MS + 1 mg/l BAP), and MS3 medium (MS + 0.5 mg/l BAP+ 0.1 mg/l NAA). The MS4 medium (5.2 shoot/explant) was the most successful in terms of the number of shoots per explant. The highest shoot regeneration from cotyledon explants was obtained from MS1 (MS + 0.5 mg/l BAP) and MS9 (MS + 2 mg/l BAP + 0.1 mg/l NAA) mediums with 91.6%. In hypocotyls explants, indirect shoot regeneration was induced in all media combinations and the rate of regeneration was found to be higher than in cotyledon explants. In root regeneration trials, 4 different indolebutyric acid (IBA) doses (0 mg/l, 0.5 mg/l, 1.0 mg/l and 1.5 mg/l) were tested on 1/2 MS medium, and 100% rooting was achieved from the control and 0.5 mg/l IBA doses.

Keywords: *Regeneration, Cabbage, Hypocotyl, Cotyledon*

Introduction

Cabbage vegetables of the *Brassicacea* family are economically significant vegetable species. This family includes approximately 350 genera and 3000 species (Şalk et al., 2008). The total production of *Brassica* group vegetables in Turkey is 935,230 t, 492,610 t of which is white head cabbage (Anonymous, 2014). Turkey is as rich in terms of its cabbage genetic resources as it is in cabbage production (Balkaya et al., 2005). Different types of cabbage are cultivated in each region in Turkey. For example leaf cabbage is cultivated in the Black Sea Region while white head cabbage is cultivated in Eastern Anatolia, and red head cabbage is cultivated in Western and Southern Regions of Turkey. The Ercis cabbage is a local head cabbage population that is cultivated in the Van province of the Eastern Anatolian Region of Turkey. Due to its loose and large head (average of 1.0 kg) and thin leaf properties it is more suited for stuffing or pickling, and is a significant source of income for the region's population.

Currently in breeding studies on *Brassica* species, biotechnological methods and genetic transformation approaches are widely used in addition to traditional plant breeding methods. However the success of these approaches depends on the use and development of an effective and suitable system of *in vitro* regeneration. Therefore studies are required to optimize the regeneration system for every species, in fact for every genotype. Cotyledon (Daud et al., 2015) and hypocotyl explants (Hazrat et al., 2007; Cristea et al., 2010; Gerszberg et al., 2015) taken from seedling cultivated *in vitro* are widely used in plant regeneration and genetic transformation studies on *Brassica* species.

Munshi et al. (2007) have reported that the highest shoot regeneration in cotyledon and hypocotyl explants taken from 7 day old *in vitro* seedlings of head cabbage (*B. oleracea* var. *capitata*) was observed on 1/2 MS containing 0.5 mg/l BA and that 98% rooting had been

achieved on 1/2 MS fortified with 0.5 mg/l IBA. In a study performed on 8 different head cabbage *(Brassica oleracea* var. *capitata)* varieties, it is stated that the highest shoot regeneration in 5 varieties was obtained from the MS + 8.88 µM BAP + 0.53 µM NAA medium in 10 day old hypocotyl and cotyledon explants cultured *in vitro*, and that hypocotyl explants had been more successful in terms of callus stimulation and organogenesis. The same researchers have also stated that rooting was achieved in all varieties and that an increase was observed in the number of healthy and strong roots in the MS + 5.37 µM NAA medium (Gerszberg et al., 2015).

Sharma et al. (2014) have tested 36 different hormone combinations in head cabbage *(Brassica oleracea* L. var. *capitata)*. They have reported that the highest shoot regeneration in cotyledon explants had been obtained from the MS + 1.5 mg/l BAP + 0.50 mg/l NAA and MS + 2 mg/l BAP + 0.25 mg/l IAA medium, and that 90% root regeneration was achieved from MS + 0.1 mg/l IBA as rooting medium. Daud et al. (2015) have used stalk, root, leaf, and leaf stem explants taken from 7 day old seedlings of head cabbage that had developed *in vitro*. They have determined that plantlets had been obtained more quickly from calluses obtained from stalk and root explants cultured in the MS + BAP (1 mg/l) + NAA (0.5 mg/l) medium. In another study that had been performed on 2 genotypes of head cabbage, it is stated that the maximum shoot regeneration in hypocotyl explants taken from 5 day old seedlings developed *in vitro* had been observed on MS containing 9.1 µM zeatin and 2.8 µM IAA (Cristea et al., 2010).

The present study was undertaken to investigate the effect of MS medium formulations and different explant types (cotyledon and hypocotyl) on shoot and root regeneration in Ercis cabbage. This is the first report on *in vitro* regeneration of Ercis head cabbage.

Material and methods

Seeds belonging to the 'Ercis cabbage population' obtained from the Ercis district of Van province in Turkey have been used in the study. The seeds were washed several times under tap water, eliminating inorganic materials from the seeds. Then they were kept for 30 seconds in 70% ethyl alcohol within a laminar flow container, washed several times with double distilled water, removing ethyl alcohol. The seeds were then kept for 15 minutes in 20% commercial sodium hypochlorite solution to which a few drops of Tween-20 had been added, and finally washed by being shaken in double distilled water 3 times, 6 minutes each time. The sterilized seeds were germinated in hormone-free MS (Murashige and Skoog, 1962) medium (30 g/l sucrose, 7 g/l agar, PH: 5.8) within 6 cm glass Petri dishes (6-7 seeds/Petri dish). Cotyledon and hypocotyl explants were excised from 7 day old seedlings, and cultured on MS containing various concentrations of BAP (0.5, 1.0, and 2.0 mg/l) and NAA (0, 0.05, and 0.1 mg/l). All the cultures were kept under a 16 h photoperiod (3000 lux, approx.) regime at 23 ± 2°C. Shoots that reach an approximate length of 3 cm were transferred to 1/2 MS rooting medium containing IBA (0, 0.5, 1.0, and 1.5 mg/l) in 4 different concentrations. 4 weeks later, the number of shoot per explant and percentage, average shoot length, the number of roots obtained per explant and percentage and the average root length was calculated in the cultures.

Shoot regeneration trials were conducted with 3 repetitions for each application, and root regeneration trials were conducted with 6 trials. Variance analysis was performed in comparisons of properties investigated in the trial. The level of statistical significance was taken as 5% in calculations, and the SPSS (ver. 13) statistics package software was used for calculations.

Results and discussion

In vitro shoot regeneration trials, cotyledon and hypocotyls explants were excised from 7 day old *in vitro* seedlings, and cultured in the MS medium in 9 different hormone combinations

containing BAP and NAA (Table 1). The swelling and growths that occurred in explants 7-9 days after being planted in the MS medium were determined as development, and the number of explants which had shown development were recorded. Shoot regeneration was achieved from all explants that had observed development and growth. Hypocotyl explants were found to be more successful than cotyledon explants in terms of explant development and shoot regeneration, except in the MS1 (0.5 mg/l BAP) and MS9 (2 mg/l BAP + 0.1 mg/l NAA) mediums. While all explants (100%) registered growth in the MS7 (2 mg/l BAP) medium in hypocotyl explants, this was followed with MS6 (1 mg/l BAP + 0.1 mg/l NAA), MS4 (1 mg/l BAP), and MS3 (0.5 mg/l BAP + 0.1 mg/l NAA) with a 91.6% percentage of regeneration. MS9 (2 mg/l BAP + 0.1 mg/l NAA) was found to be the least successful medium with a 58.3% rate of regeneration.

In cotyledon explants, the highest shoot ratio was obtained in MS1 (0.5 mg/l BAP) and MS9 (2 mg/l BAP + 0.1 mg/l NAA) mediums with a 91.6% rate of regeneration, and this was followed by MS4, MS5, and MS8 in that order. MS6 and MS7 mediums were found to be least successful in terms of shoot regeneration with a 41.6% rate of regeneration. Indirect shoot regeneration was occurred in all medium combinations in hypocotyl explants (Figure 1). The rate of callus formation ranged between 41.6 – 100% in hypocotyl explants, and between 0-33.3% in cotyledon explants (Table 2).

Table 1. Effect of BAP and NAA in the MS medium on the number of shoots per explants, regeneration rate and on shoot length.

Medium	Hormone (mg/l)		Shoot number/explant and regeneration rate (%)				Shoot length (cm)	
	BAP	NAA	Hypocotyl	R.R (%)	Cotyledon	R.R (%)	Hypocotyl	Cotyledon
MS1	0.5	0	3.16 bc	83.3	2.92 ab	91.6	1.83 bc	2.33 a
MS2	0.5	0.05	3.75 b	83.3	0.66 c	50.0	3.80 a	1.60 ab
MS3	0.5	0.1	4.42 ab	91.6	0.75 c	58.3	2.60 ab	1.60 ab
MS4	1.0	0	5.25 a	91.6	2.58 b	83.3	2.0 bc	1.63 ab
MS5	1.0	0.05	2.42 cd	83.3	1.16 c	75.0	1.53 bc	1.66 ab
MS6	1.0	0.1	4.33 ab	91.6	0.41 c	41.6	2.60 ab	1.0 b
MS7	2.0	0	4.33 ab	100	0.75 c	41.6	2.03 bc	1.40 ab
MS8	2.0	0.05	2 .33 cd	75.0	1.36 c	66.0	1.83 bc	2.0 ab
MS9	2.0	0.1	1.16 d	58.3	3.75 a	91.6	1.0 c	2.26 a
LSD.05			**1.21**		**1.09**		**1.28**	**1.03**

R.R.: Regeneration rate

While the highest number of shoots per explant was obtained from the MS4 (5.25 shoot/explant) medium in hypocotyl explants, this was followed by the MS3 (4.42 shoot/explant), MS6 (4.33 shoot/explant), and MS7 (4.33 shoot/explant) mediums that are included in the same statistical group, in that order. The MS9 medium was found to be the least successful environment in terms of the number of shoots per explants (Table 1). In many studies conducted on cabbage, it is reported that the composition of the medium and type of explant are influential on shoot regeneration, and that hypocotyl explants yield more successful results as compared to other explant types in terms of the rate of shoot regeneration (Munshi et al., 2007; Dai et al., 2009; Ravanfar et al., 2009; Pavlovic et al., 2010). Figure 1 shows shoot formation from cotyledon and hypocotyl explants.

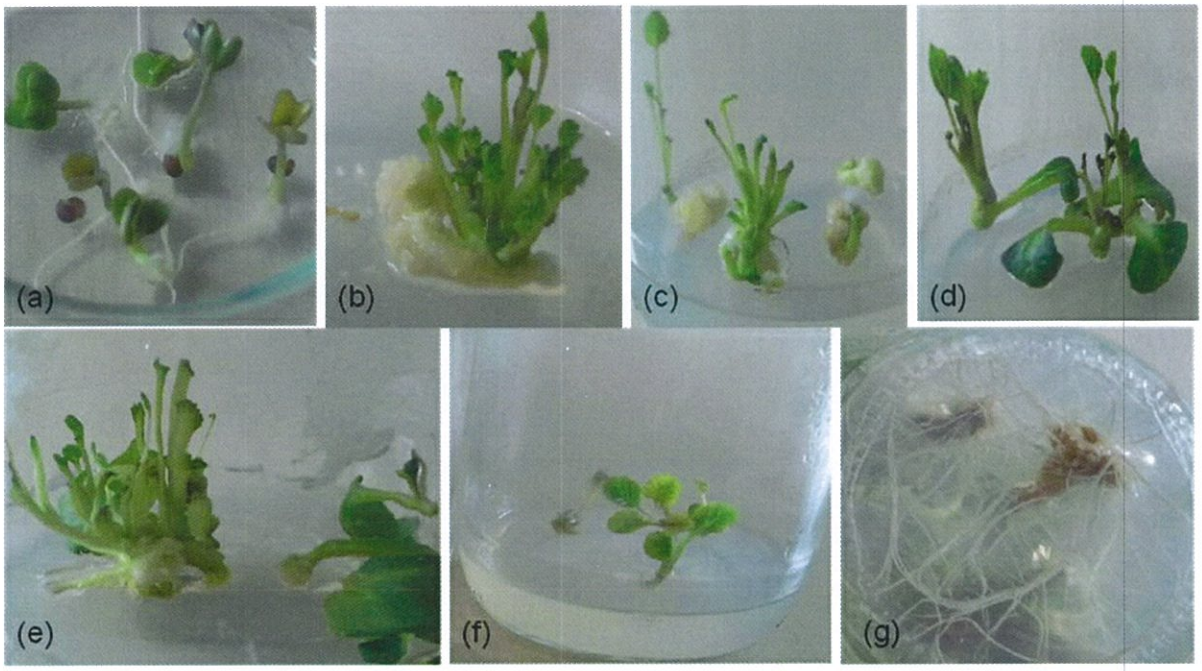

Figure 1. In Ercis head cabbage, (a) 7 day old in vitro germinated seedlings, (b, c) indirect shoot formation from hypocotyl explants, (d, e) direct shoot formation from cotyledon explants, (f) plants transferred to rooting medium, (g) root formations in rooting medium.

Table 2. Effect of BAP and NAA in the MS medium on the rate (%) of callus formation

Medium	Hormone (mg/l)		Callus formation rate (%)	
	BAP	NAA	Hypocotyl	Cotyledon
MS1	0.5	0	66.6	0.0
MS2	0.5	0.05	91.6	16.6
MS3	0.5	0.1	100.0	16.6
MS4	1.0	0	100.0	0.0
MS5	1.0	0.05	91.6	0.0
MS6	1.0	0.1	66.6	8.3
MS7	2.0	0	58.3	25.0
MS8	2.0	0.05	41.6	33.3
MS9	2.0	0.1	50.0	33.3

The results of our study also have been found to support this literature, and more successful results have been obtained from hypocotyl explants in terms of shoot regeneration. A statistically significant decrease has been determined in the number of shoots obtained from cotyledon explants. While the highest number of shoots was obtained from the MS9 (3.75 sürgün/eksplant) medium, this was followed by the MS1 (2.92 shoot/explant) and MS4 (2.58 shoot/explant) mediums. The number of shoots that was obtained from other medium combinations were found to be low, and the differences between them were found to be statistically insignificant (Table 1). It has been determined that various doses of are BAP (1.0 – 3.5 mg/l) and NAA (0.1-0.5 mg/l) are generally used in shoot regeneration studies in studies conducted on various *Brassica* species (Basak et al, 2012; Sharma et al, 2014). Shoot length

ranged between 1.0 – 3.8 cm in shoots developing from hypocotyl explants, and 1.0 – 2.33 cm in shoots developing from cotyledon explants (Table 1).

Literature scans performed have indicated that various doses of IBA (0 – 1.0 mg/l) or NAA (0.1 – 4.0 mg/l) are generally used as rooting medium on *Brassica* species, and that the results obtained are successful (Munshi et al, 2007; Ravanfar et al., 2009; Sharma et al., 2014; Daud et al., 2015; Gerzberg et al, 2015). In our study, considering these literature statements, the shoots obtained were transferred to rooting mediums containing various doses of IBA (0 mg/l, 0.5 mg/l, 1.0 mg/l, and 1.5 mg/l) in a 1/2 MS medium. The impact of various concentrations of IBA on the number of roots obtained per shoot, root length, and the rate of regeneration are shown in Table 3. The highest number of roots per shoot (14.16 root/shoot) was obtained from the 0.5 mg/l IBA dose. The number of roots per shoot decreased in other doses, while the differences between the doses were found to be statistically insignificant (Table 3). While a higher result in terms of root length was obtained from the control group to which no hormones had been added, no positive effects on root length of increased IBA doses were found.

Table 3. Effect of various concentrations of IBA in the 1/2 MS medium on rooting

Hormone (mg/l) IBA	**Root number/shoot**	**Root length (cm)**	**Regeneration rate (%)**
0	8.50 b	2.35 a	100
0.5	14.16 a	1.82 ab	100
1.0	8.30 b	1.78 ab	83.3
1.5	8.16 b	1.56 b	66.6
LSD.05	**1.84**	**0.66**	

Conclusion

It has been concluded that in future studies that will be conducted on *in vitro* shoot regeneration in the 'Ercis' head cabbage genotype, it would be beneficial to use hypocotyl explants taken from 7 day old sterile seedlings, and make trials with a higher number of repetitions using 0.5 – 2.0 mg/l doses of BAP in the MS medium. In terms of *in vitro* rooting, it is recommended that no hormones be added to the nutrient medium, or that a dose of 0.5 mg/l IBA not be exceeded in future studies.

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CERTIFICATE
of attendance

This certificate guarantees that

Burcu TUNCER

has attended

VII International Scientific Agriculture Symposium "Agrosym 2016"
that has been held from 06th to 09th October on Jahorina mountain,
Bosnia and Herzegovina, and presented her paper entitled:

IN VITRO SHOOT AND ROOT REGENERATION IN ERCIS CABBAGE (BRASSICA OLERACEAE VAR. CAPITATA)

Academician Dusan Kovacevic
President of Scientific Committee

Prof. dr Vesna Milic
President of Organization Committee

AgroSym 2016

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East Sarajevo, 8th May 2016

Burcu Tuncer
Yuzuncu Yil University, Agricultural Faculty, Department of Horticulture, Van, Turkey

Subject: Invitation/acceptance letter

Dear Burcu Tuncer,

We hereby have the honour and pleasure of inviting you to our Symposium. Please consider this letter as an official invitation to facilitate the processing of any visas documents needed to enable you to attend the 7th International Scientific Agriculture Symposium „Agrosym 2016" which will be held on Jahorina mountain (Bosnia and Herzegovina), 6-9 October 2016.

The aim of this event is to promote scientific - research work in areas of Agriculture, Food, Rural Development, Environmental protection and Forestry.

I am pleased to inform you that the scientific committee of the Symposium has accepted your contribution titled: *"IN VITRO SHOOT AND ROOT REGENERATION IN ERCİS CABBAGE (BRASSİCA OLERACEAE VAR. CAPITATA)"* to be presented as oral presentation at the Symposium.

We look forward to welcoming you in Bosnia!

Sincerely yours,

General Secretary of the Organizing Committee
Dr Sinisa Berjan

Синиша Берјан



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