International Journal of Agriculture Sciences
ISSN: 0975-3710&E-ISSN: 0975-9107, Volume 8, Issue 58, 2016, pp.-3247-3251.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

THE EFFECTS OF MEDIA COMPOSITION ON *IN VITRO* GYNOGENIC EMBRYO INDUCTION IN *Allium tuncelianum* (KOLLMAN) ÖZHATAY, MATTHEW, ŞİRANECİ)

YARALI FAIKA*[1] AND YANMAZ RUHSAR[2]
[1]Department of Horticulture, Faculty of Agriculture, Unıversity of Kilis 7 Aralik, Kilis, 79000, Turkey
[2]Department of Horticulture, Faculty of Agriculture, Unıversity of Ankara, Ankara, 06110, Turkey
*Corresponding Author: Email-faikayarali@gmail.com

Received: November 11, 2016; Revised: November 22, 2016; Accepted: November 23, 2016; Published: November 30, 2016

Abstract- *Allium tuncelianum* is utilized as garlic in Eastern region of Turkey. There have been conducted out some studies for propagation of *Allium tuncelianum* but there is not any breeding material yet. It is the first research about determining of gynogenic induction capacity of *Allium tuncelianum* (Kollman) Özhatay, Matthew, Şiraneci) by using flower bud culture. To determined effect of plant growth regulators on gynogenesis induction, 1 mg/l and 2 mg/l of 2,4-D and BAP and their combinations were supplemented in BDS medium was. Induction medium including different combinations of auxin (2,4-D) and cytokinin (BAP) were effective on callus development on explants. The highest callus formation rates were obtained from 2+1 mg/l 2,4-D+BAP and 2+2 mg/l 2,4-D+BAP combinations. It was provided the development of the flower buds in induction medium and callus has formed at 55,28% of flower buds but plantlets could not be achieved from callus. Therefore, it is thought to be more obscurity on gynogenesis induction of *Allium tuncelianum*. Haploid plant could not be obtained in this research but this study is important due to the guidance for future studies.

Keywords- *Allium tuncelianum*, gynogenesis, 2,4-D, BAP

Citation: Yarali Faika and Yanmaz Ruhsar (2016) The Effects of Media Composition on *In-Vitro* Gynogenic Embryo Induction in *Allium tuncelianum* (Kollman) Özhatay, Matthew, Şiraneci). International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 8, Issue 58, pp.-3247-3251.

Academic Editor / Reviewer: Dr Disharee Nath

Introduction

The genus *Allium* belongs to *Alliaceae* family has a diverse taxon comprising of over than 700 species and 164 of these species has been originated from Turkey. Forty percent of *Allium* species found in Turkey are endemic [1,2]. *Allium tuncelianum* which is utilized as garlic in Eastern region of Turkey might be the wild ancestor of garlic. *A. tuncelianum* is originally named as *Allium macrochaetum* Boiss and Haussk subsp. tuncelianum Kollmann [3]. Although, it is native to 'Tunceli' province (especially at Platos of Munzur Mountains in Ovacik district) of Turkey [2,4-7]. It is locally called as 'Tunceli garlic' or 'Ovacik garlic' in the region becuse of its resemblance to common garlic and grows in limited area especially Ovacik and Pülümür district [2,8,9]. *Allium tuncelianum* have single cloved white bulb different from common garlic, which has a multiple cloved bulb and it can be easily propagated with seeds and bulbs. It has lower number of peel (1-2 peel) than common garlic and it can also produce fertile flowers with white-to-purple flowered inflorescences and seeds [5,6,9-11]. Seed of *Allium tuncelianum* is fertile and black colour that can be used for propagation [2,11,12]. *Allium tuncelianum* stimulates the body's immune system, lowers the level of sugar and cholesterol in the blood, improve blood circulation thus reduces the risk of heart attack [2, 13]. Approximately, 15-25 tons of Tunceli garlic has been collected from nature for domestic and medical purpose by herbalists in Turkey [2, 14]. Picking of endangered geophytes for trade is banned in Turkey for conservation purpose in agreement with Convention on the International Trade in Endangered Species (CITES). There is a need to develop strategies to conserve *Allium tuncelianum* before its extinction [2, 6]. There have been conducted out some studies for propagation of *Allium tuncelianum* but there is not any breeding material yet. Today, biotechnological breeding methods offers great benefits in shortening of breeding duration and obtaining inbred lines used in hybrid breeding in a short time. Using dihaploidization techniques that allow shortening the breeding period were obtained doubled haploids in other *Alliums* such as onion, garlic and leek [15- 20). Different methods have been improved for *in vitro* onion haploid production, but only gynogenesis has been reported to be successful in *Alliums*. Haploid onion plants has been produced by gynogenesis from ovules, ovaries or whole flower buds [16,17,21-34]. Studies on gynogenesis in onion showed that embryo induction rate of ovary and flower bud culture was higher than ovule culture [16].There are no studies on *in vitro* gynogenic induction in *Allium tuncelianum* (Kollman) Özhatay, Matthew, Şiraneci). In this study, it was aimed to obtain haploid plants via gynogenesis using flower bud culture in *Allium tuncelianum* which is important endemic plant of Turkey. To ensure optimization technique of our own laboratory conditions it was used successful techniques that previous studies indicated in other *Allium* species.

Materials and Methods

This study was carried out in 2012. Bulbs of *Allium tuncelianum* (Kollman) Özhatay, Matthew, Şiraneci) were provided from province Tunceli in Turkey [Fig-1] and were planted in Vegetable Research and Application Garden of Horticulture Department of Agriculture Faculty, University of Ankara at date of 01 November 2012. In order to encourage the development of plants, 350-400 g/l of fertilizer (Novachem 18-18-18+ME) was applied with irrigation during vegetation. 620 bulbs of *Allium tuncelianum* were planted in prepared plot with distance of 30x15 cm [Fig-2].

The composition of the induction medium

There is no data about studies in vitro gynogenic induction of *Allium* tuncelianum that's why BDS [16, 17, 23, 30, 31, 36, 37] medium has been found successful for induction of gynogenic embryos in *Allium* species was chosen as induction medium [35].

BDS medium was supplemented with 1-2 mg/l of 2,4-dichloro-phenoxyacetic acid (2,4-D) and 1-2 mg/l of 6-benzylaminopurine (BAP) and their combinations. To determine the effect of sucrose on gynogenic induction on *Allium tuncelianum*, 5% and 10% doses of sucrose were used as carbohydrate source in induction medium. All media were adjusted to pH 6.0 and were solidified with 7.2 g/l phytagar [14, 38].

Fig-1 Bulbs of *Allium tuncelianum* (Kollman) Özhatay, Matthew, Şiraneci)

Fig-2 Plants of *Allium tuncelianum* (Kollman) Özhatay, Matthew, Şiraneci) at stage of flowering.

Sterilization of flower buds and culture media

Unopened flower buds were taken 3 or 4 days before anthesis from donor plants grown in the field [Fig-3]. Whole umbels or individual flower buds were dipped in 70% ethanol for 3 min, 15% Clorox ((0.9% sodium hypochlorite) + 0.1% Tween-20) for 30 min with stirring for disinfection and rinsed three times with sterile double distilled water [17]. Media were sterilized by autoclaving for 20 minutes at 121^{0}C.

Fig-3 Flower buds of *Allium* tuncelianum (Kollman) Özhatay, Matthew, Şiraneci)

Culturing the flower buds

Explants that used in flower bud culture were prepared by cutting stalks of flower buds for flower bud culture. Flower buds were placed in petri plates containing 20 ml of induction medium (25 explants/plate) at laminar flow cabinet in sterile conditions. Petri dishes were sealed with stretch film and cultured at 25 °C under cool white fluorescent (6000 lux) with 16h light/8h dark photoperiod. Total 5000 flower buds were cultured in induction medium [Fig-4]. Developing explants were transferred to B1 medium supplemented with 1 mg/l of NAA (Naphthaleneacetic acid) and 2mg/l of 2iP (Isopentyladenine) and 100 g/l sucrose 6–7 weeks after initial culture on BDS medium [Fig-5].

Fig-4 a: Flower buds of *Allium tuncelianum* in induction medium b: The petri dishes were taken into climate chambers after the planting of flower buds.

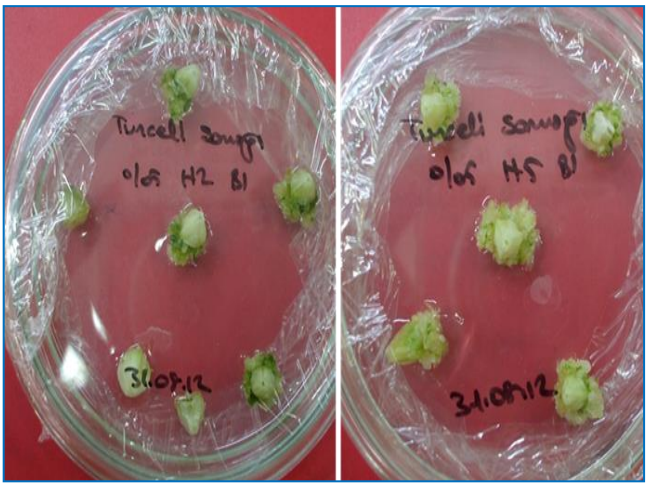

Fig-5 Developing flower buds of *Allium tuncelianum* of in medium B1

Statistical Analysis

Developing cultures have been observed after a week after of initiation. Development stages such as swelling and callus formation were determined on flower buds. In addition, number and vitrification rate were determined on explants. Number of explants forming callus (number/petri) rate of callus formation (%) were calculated. All experiments were established according to completely randomized design (CRD) with twenty replicates in order to reveal the relationship composition of medium and sucrose doses. Turkey test was used to identify the different groups after the variance analysis. The level of statistical significance was taken as 5% and calculations were made with JMP software package version 5.0.1.

Results and Discussions

The effect of sucrose dose

The effect of sucrose doses on callus formation on flower buds in induction medium was statistically significant according to the result of analysis of variance. Callus formation rate was %58,24 and %52,52 at respectively at dose of 5% and %10 sucrose [Table-1].

Table-1 *The effect of sucrose dose on callus formation (number/petri dish)*

Sucrose dose (%)	Planted explant number	Number of explants forming callus	Callus formation rate (%)
5	25	14,56a	58,24
10	25	13,13b	52,52

*Significant at 5 percent level of significance.

Swelling rate and vitrification rate of flower buds in induction medium also affected by sucrose dose on. The highest swelling rate (%87,84) on explants obtained from medium added %5 sucrose. Vitrification rate has increased due to an increase in the dose of sucrose. The highest vitrification rate (%22,60) was obtained from medium added %10 sucrose dose [Table-2].

Table-2 *The effect of sucrose dose on swelling and vitrification of flower buds from Allium tuncelianum*

Sucrose dose (%)	Swelling explant number	Swelling explant rate (%)	Vitrificated explant number	Vitrificated explant rate (%)
5	2196	87,84	330	13,20
10	1906	76,24	565	22,60

The effect of media composition

The effect of media copmposition on callus formation on flower buds in induction medium was statistically significant according to the result of variance analysis. It was determined that callus formation rate increased with increasing doses of auxin and cytokinin compared with conrtol medium has no auxin and cytokinin. The highest callus formation rate (73,28%) was obtained from media suplemented with 2 mg/l of 2,4-D and 2 mg/l of BAP [Table-3].

Table-3 *The effect of media composition on callus* formation *on flower buds from Allium tuncelianum (number/petri dish)*

Media composition	Number of explants forming callus	Callus formation rate (%)
0+0 mg/l 2,4-D+BAP	7,69c	30,76
1+1 mg/l 2,4-D+BAP	12,63b	50,52
1+2 mg/l 2,4-D+BAP	14,03b	56,12
2+1 mg/l 2,4-D+BAP	16,56a	66,24
2+2 mg/l 2,4-D+BAP	18,32a	73,28

*Significant at 5 percent level of significance

Callus formation and development of flower buds of *Allium tuncelianum* (Kollman) Özhatay, Matthew, Şiraneci) is shown in [Fig-6].

Fig- 6 Callus formation on flower bud of *Allium tuncelianum*

Media composition effected to swelling rate on flower buds in induction medium [Table-4]. The highest swelling rate (95,70%) was determined on explants in medium suplemented with 2 mg/l of 2,4-D and 1 mg/l of BAP. Effect of medium composition on vitrification has not been significant. Vitrification rate was ranged between 13,40% and 23,30%.

Table-4 *The effect of media composition on swelling and vitrification on flower buds from Allium tuncelianum*

Media composition	Swelling explant number	Swelling explant rate (%)	Vitrificated explant number	Vitrificated explant rate (%)
0+0 mg/l 2,4-D+BAP	634	63,40	134	13,40
1+1 mg/l 2,4-D+BAP	814	81,40	162	16,20
1+2 mg/l 2,4-D+BAP	808	80,80	172	17,20
2+1 mg/l 2,4-D+BAP	957	95,70	233	23,30
2+2 mg/l 2,4-D+BAP	889	88,90	194	19,40

In this experiment, callus formation occurred on flower buds were from *Allium tuncelianum* (Kollman) Özhatay, Matthew, Şiraneci) in induction medium. Any shoot or root development from callus was not observed in subcultures during approximately 3 months. *Allium tuncelianum* (Kollman) Özhatay, Matthew, Şiraneci) is one of the endemic plants in Turkey and has not been any previously studies on the its gynogenesis frequency. For this reason determining gynogenesis capacity of *Allium tuncelianum* was aimed from flower bud culture in this research. Different factors such as genetic factors, including cultivar, donor plant genotype and geographic orijin are thought to be the most important factors for the success of gynogenesis induction [16, 22, 39, 40). Gynogenesis response of *Allium* species depended on the culture media which has been used. Also, media composition requirements may be different for *Allium* species and varieties to obtain gynogenic haploid plant [20, 25, 41). Plant growth regulators supplemented to the culture medium have also significant effect on gynogenesis [20, 25, 42]. It is reported that supplementation of different doses of 2,4-D (2,4-Dichlorophenoxyacetic acid) as auxin and BAP (Benzylaminopurine) as cytokinin in nutritional medium increasegynogenic haploid induction in onion [17, 30, 31, 33, 34, 40-47]. Absence of a study conducted on gynogenesis capacity of *Allium tuncelianum* previous literatures focused on onion, it was tried 1 and 2 mg/l of 2,4-D and BAP combinations in induction medium. Explant development frequency had been very low or absent in induction medium without auxin and cytokinin. Whereas it was found that the growing number of explants increased with increasing doses of auxin and cytokinin. Combinations of 2+1 mg/l of 2,4-D+BAP ve 2+2 mg/l of 2,4-D+BAP were more effective than other doses on growing flower buds [39, 43, 45]. To determine effect of sucrose, source of carbohydrates in culture medium on gynogenic embryo induction different sucrose doze; %5 and %10 were used. Developed explants were evaluated in the induction medium in experiment. A total of 5,000 flower buds planted in induction medium have different sucrose doses. The highest callus formation rate from flower buds were determined 58.24% and 52.52% respectively 5% and 10% of sucrose dose. It was determined that 5% sucrose dose was more effective on the formation of callus. Therefore dose of 5% sucrose could be suggested in future studies. Sucrose dose was effective on vitrification on flower buds. While vitrification rate was determined as 22,60% at induction medium with 10 % of sucrose dose 13,20% of flower buds in inducton medium with 5% of sucrose doses were observed vitrification. It is reported that high amount of carbohydrates and auxin- cytokinin in culture medium may cause vitrification on explants [48, 49]. Smilar results were observed in our study and vitrification rate was higher in induction medium with 10% of sucrose dose. Vitrification rate on flower buds was influenced by the amount of auxin and cytokinin in induction medium. Compared with medium have no auxin cytokinin, it was determined that the vitrification rate of explants increased with increasing doses of auxin and cytokinin. The vitrification rate was

ranged between 13,40% ile 23,30% and the highest rate was obtained from media suplemented with 2 mg/l of 2,4-D and 1 mg/l of BAP.

Conclusion

There is no research on haploid plant production in *Allium tuncelianum* (Kollman) Özhatay, Matthew, Şiraneci) via gynogenesis. This paper is the first study on gynogenesis by flower bud culture in *Allium tuncelianum* that is a specific genetic resources of Turkey. According to the results, 2 mg/l of 2,4-D and 1 mg/l of BAP plant growth regulator combinations were found effective on callus formation on explants. It was provided the development of the flower bud in induction medium and callus has formed at 55,28% of flower buds but plantlets could not be achieved from callus. Therefore, it is thought to be more obscurity on gynogenesis induction of *Allium tuncelianum*. So, our goal is to solve these problems in future studies. As a result, it is recommended that future studies will be planned with the higher dose of plant grow regulators.

Author Contributions: The paper is a part of the PhD thesis of the first author under the research guidance of the second author.

Abbreviations: BAP 6-benzylaminopurine ; CRD Completely randomized design

Conflict of Interest: None declared

References

[1] Song S.I., Cheong J.J. and Choi Y.D. (2007) *Biotechnology in Agriculture and Forestry,* 59, Transgenic Crops IV.

[2] Aasım M. (2015) *Romanian Biotechnological Letters,* 20(5), 10845-10851.

[3] Etoh T. and Simon P.W. (2002) Diversity, fertility and seed production of garlic, H.D. RABINOWITCH, L. CURRAH, eds., *Allium* Crop Science: Recent Advances, CABI Publishing, New York, 2002, pp. 101–117

[4] Baktır I. (2005) *Tarim Kongresi,* 206-208 pp.

[5] Yanmaz R. and Ermiş S. (2005) Tunceli sarımsağı (*Allium tuncelianum* Kollman, Özhatay,. Matthew, Şiraneci)) tohumlarındaki çimlenme probleminin çözülmesi üzerine araştırma. Türkiye 2. Tohumculuk kongresi, Adana, s: 101-106.

[6] Yanmaz R., Ezgi Y.E., Kantoğlu K.Y. and Alper A. (2010) *Journal of Food, Agriculture and Environment,* 8, 572–576.

[7] Baktır I., Yılmaz G., Göktürk R.S. and Karagüzel O. (2013) *Acta Hortic.,* 1002, 297-300. DOI: 10.17660/Actahortic.2013.1002.38. Http://Dx.Doi.Org/10.17660/Actahortic.2013.1002.38

[8] İpek M., İpek A. and Simon P.W. (2008) *Scientia Horticulturae,* 115, 409–415.

[9] Kiralan M., Rahimi A., Arslan N. and Bayrak A. (2013) *Journal of Essential Oil Bearing Plants,* 16(3), 417-420.

[10] Taşkın H., Baktemur G., Kurul M. and Büyükalaca S. (2013) *Hindawi Publishing Corporation he ScientificWorld Journal,* Article ID 781282, 5 pages, http://dx.doi.org/10.1155/2013/781282.

[11] Kızıl S., Icgil D.Y. and Khawar K.M. (2014) *Journal of Horticultural Science & Biotechnology,* 89(4), 408–414.

[12] Arslan N. (2013) Tunceli sarimsagi (*Allium tuncelianum*). http://www.gidahatti.com/k-sa-k-sa-duenyadan-arsivi/tuncelisar-msag-allium-tuncelianum. 1 March 2014.

[13] Koşar M., Koyuncu M. and Başer K.H.C. (2005) Folk use of some wild and cultivated *Allium* species in Turkey. Proceedings of the IVth International Congress of Ethnobotany (ICEB 2005), Istanbul, Turkey.

[14] Fırat M. (2015) *Manas Journal of Agriculture and Life Science MJAL,* 5(1), 80–86.

[15] Keller E.R.J. and Korzun L. (1996) Haploidy in onion (*Allium cepa* L.) and other *Allium* species. *In vitro* haploid production in higher plants. Chapter. Edit: Jain SM, Sopory SK and Veilleuks RE, Kluwer Academic Publisher, 3, p: 51-75. Netherlands.

[16] Bohanec B. and Jakse M. (1999) *Plant Cell Reports,* 18, 737-742.

[17] Alan A.R., Mutschler M.A., Brants A., Cobb E. and Earle E.D. (2003) *Plant Science,* 165(6), 1201-1211.

[18] Palmer C.E.D. and Keller W.A. (2005) Overview of haploidy. Biotecnology in Agriculture and Forestry, Haploids in Crop Imprevement II (Ed. By Palmer CE, Keller WA and Kasha KH), 56, pp:3-7.

[19] Forster B.P., Bors E.H., Kasha K.J. and Touraev A. (2007) *Trends in Plant Science,* 12(8), 368-375.

[20] Murovec J. and Bohanec B. (2012) Haploids and doubled haploids in plants breeding. Agricultural and Biological Sciences, Chapter 5, pages: 87- 106. Edited by Ibrokhim Y. Abdurakhmonov, ISBN 978-953-307-932-5.

[21] Muren R. (1989) *HortScience,* 24(5), 833- 834.

[22] Campion B. and Alloni C. (1990) *Plant Cell, Tissue and Organ Culture,* 20(1-6), Netherlands.

[23] Keller J. (1990a) Euphytica, 47, 241-247.

[24] Keller J. (1990b) *Archiv für Züchtungsforschung,*20(3),pp:189-197.

[25] Mukhambetzhanov S.K. (1997) *Plant Cell, Tissue and Organ Culture,* 48, 111-119.

[26] Javornik B., Bohanec B. and Campion B. (1998) *Plant Breeding,* 117(3), 275-278.

[27] Hassandokht M.R., Kashi A., Campion B. and Bozorgipour R. (2000) *Seed and Plant,* 16(3), 300-312.

[28] Jakse M., Bohanec B. and Havey M.J. (2002) Genotypic and environmental effects on gynogenic haploid induction in onion. Meeting Abstract. Publisher: Faculty of Agriculture, Ain Shams University.

[29] Szulc S.V., Pedzinski M., Matuszak W., Majchrzak M. and Rubik E. (2002) Gynogenesis ability in onion breeding at, Spojnia ltd., Nochowo. Number: 488, pages: 323-331.

[30] Alan A.R., Brants A., Cobb E., Goldschmied A., Mutschler M.A. and Earle E.D. (2004) *Plant Science,* 167(5), 1055-1066.

[31] Musial K., Bohanec B., Jakse M. and Przwara L. (2005) *Biol-Plant,* 41, 446-452.

[32] Geoffriau E., Kahane R. and Tanguy J.M. (2006) *Physiologia Plantarum,* 127, 119-129.

[33] Sulistyaningsih E., Aoyagi Y. and Tashiro Y. (2006) *Plant Cell Tissue and Organ Culture,* 86, 249-255.

[34] Forodi B.R., Hassandokht M., Kashi A. and Sepahvand N. (2009) *Horticulture Environment and Biotechnology,* 50(5), 461-466.

[35] Dunstan D.I. and Short K.C. (1997) *Physiol Plant,* 41: 70-72.

[36] Puddephat I.J., Robinson H.T., Smith B.M. and Lynn J. (1999) *Plant Cell, Tissue and Organ Culture,* 57, 145-148.

[37] Cho K.S., Hong S.Y., Yun B.Y., Kwon Y.S. and Huh E.J. (2006) *Hort Environ. Biotechnol,* 47(3), 110-116.

[38] George E.F., Hall M.A. and Klerk G.J. (2008) The components of plant tissue culture media II. Organic additions, osmotic and pH effects and support systems. Plant Propagation by Tissue Culture 3rd Edition, Chapter 4. P: 115-173.

[39] Michalik B., Adamusa A. and Nowak E. (2003) *Plant Science,* 165(6), 1201-1211.

[40] Chen J.F., Cui L., Malik A.A. and Mbira K.G. (2011) *Plant Cell Tiss Organ Cult,* 104, Pp: 311-319. DOI 10.1007/s11240-010-9874-6.

[41] Yaralı F. and Yanmaz R. (2013) *Türk Bilimsel Derlemeler Dergisi,* 6(2), 44-50.

[42] Bohanec B. (2009) Doubled haploids via gynogenesis. Advances in haploid production in higher plants. Ed.: Forster, Brian P., Touraev, Alisher, Jain, S. Mohan, Springer Science Business Media B.V., Page: 35-46.

[43] Campion B., Azzimonti MT., Vicini E., Schiavi M. and Falavigna A. (1992) *Plant Science,* 86(1), 97-104.

[44] Geoffriau E., Kahane R. and Rancillac M. (1997) *Euphytica,* 94, 37-44.

[45] Martinez L.E., Agüero C.B. and Galmarini C.R. (1997) *Acta Hort. (ISHS),* 433, 447-454.

[46] Bekheet S.A. (2004) *Arab Univ. J. Agric. Sci., Ain Shams Uiv.,Cairo,*12(2), 721-733.

[47] Reed S.M. (2005) Haploid cultures, Plant Development and Biotechnology, Chapter 17. Pages: 225-234.
[48] Paques M. and Boxus P.H. (1987) *Acta Hort. ISHS),* 212,155-166.
[49] Ziv M. (1991) *Micropropagation,* pp:45-69.