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Research article

AN EFFICIENT METHOD FOR MICROPROPAGATION OF GARLIC (*ALLIUM SATIVUM* L.). BY SOMATIC EMBRYOGENESIS AND ARTIFICIAL SEED PREPARATION WITH SOMATIC EMBRYO

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ABSTRACT: Micropropagation is the scientific method for *in vitro* synthesis of plant. This research used to achieve and develop embryo induction and the multiple shoots initiation, roots proliferation from the embryo itself of *Allium sativum* L. (belong to the family Alliaceae). Somatic embryos were regenerated from callus. Embryo size was maximum in Ms Medium containing 0.5 mg/lKn + 0.25 mg/l 2, 4-D. The induction of multiple shoots from embryo was highest in MS medium supplemented with 1 mg/l Kn. For rooting different concentration of IBA were used and highest rooting was recorded on MS medium supplemented with 1.0 mg/l IBA. Fully mature somatic embryo of *Allium sativum* were used for the purpose of artificial seeds formation. Best seeds were prepared by using 3% sodium alginate and 1.1% $CaCl_2$.

Key Words: Somatic embryo, *Allium sativum L.*, Shoot initiation, Root proliferation.

INTRODUCTION

Allium sativum L., commonly known as garlic, belongs to a member of the onion family (Alliaceae). Garlic has beenused throughout the ages for both culinary and medical purpose. Extensive research work has been carried out on the health promoting and medicinal properties of garlic. *A.sativum* has shown a variety of biological activities including antioxidant, cancer prevention, liver protection, immunomodulation and reduction of cardiovascular disease risk factors[1]. Garlic is characterized by medicinal properties due to the content of over 2000 biologically active compounds [2]. Garlic has an unusually high concentration of sulfur containing compounds. Sulfur compounds, including allicin (thio-2-propene-1-sulfinic acid S-allyl ester) were confirmed to be the main active components in the root bulb of the garlic plant [3]. Allicin has the wide range of biological and pharmacological activities, such as anticoagulation, antihypertensive, antimicrobial, antibiotic, antiparasitic, antimycotic, antiviral, antitumoral, anti-oxidant, anti-aging, antiplatelet, detoxifies heavy metals, fibrinolusis, hypolipidaemic (lipid-lowering) and immune enhancer and modulator [4] .

The micropropagation of garlic is by division of the individual cloves of its bulbs. Because garlic almost never produces fertile seeds, it must be propagated vegetatively. As the garlic is vegetatively propagated, the health status of the crop is affected by both primary and secondary virus infection which accumulates in each crop cycle. Almost all garlic seed used is contaminated with one or more pathogens, mainly viruses that play a main role in yield reduction and quality, also reducing the storage longevity of the harvested bulbs .With the aim to improve the health quality of garlic seeds, virus-free stocks tissue culture is considered as an alternative tool. Therefore, the use of shoot meristem with basal portion as explants for micropropagation of garlic is more suitable than other source of explants.

MATERIAL AND METHODS

Invitro grown callus of *Allium sativum* collected from Vital Biotech, Kota, Rajasthan. Experiments were repeated for somatic embryos development on containing 3% sucrose was solidified with 0.8% agar (Qualigens). The *p*H of the media was adjusted to 5.9±0.02 with 1 N NaOH or 1 N HCl solutions prior to autoclaving. Media poured in culture vessels were steam sterilized by autoclaving at 121°C and 15 psi for 15-20 min.

The cultures were incubated under controlled Conditions of temperature (25±2°C), light (2000- 2500 lux for 16 h/d provided by fluorescent tubes) and 60-70% humidity. For each experiment a minimum of 7 replicates were taken and experiments were repeated thrice. Callus implanted vertically on MS medium prepared with specific concentrations of BAP, Kn with 2, 4-D were used for the formation of somatic embryos.

Observations were recorded after an interval of 4 wk. Once culture conditions for embryo induction from callus were established, then the embryo produced *in vitro* were sub cultured on fresh medium.

The embryo pieces were inoculated in various concentrations of BAP and Kn. Among these, the maximum number of shoots (3.42±0.39) and maximum shoot length (7.54±0.31cm) were developed on MS media fortified with 1 mg/l Kn. For the multiple shoot proliferation *in vitro* grown embryos were sub cultured in the different concentrations of BAP, Kn with 2,4-D.

Rooting beneath somatic embryos attempted under *in vitro* conditions. Auxins (IBA) alone in different concentrations (0.5-2.5 mg/l) were incorporated in the agar (0.8%) solidified medium containing 1/4 MS salts and 1.0% sucrose.

Embryos were used for the preparation of artificial seeds. *In vitro* regenerated somatic embryos and regenerating calli were used in the present study as propagules for encapsulation. The propagules were separated aseptically and placed in a gel matrix containing MS salts and 3% sodium alginate. The explants along with the medium were dropped into a solution of calcium chloride (1.1%) using a pipette or a glass tube. After 40 min the beads were recovered by decanting the $CaCl_2$ solution and washed in 3-4 changes of sterile water.

The synthetic seeds were stored in 250 ml flasks sealed with aluminium foil and different media like sterile water, MS basal medium with or without growth regulators were used to soak these seeds to avoid drying. The flasks were incubated at three different temperatures (5^0C, 15^0C, 22^0C) to study the effect of temperature on storage.

RESULTS AND DISCUSSION

The callus when inoculated on MS medium containing BAP and Kn with 0.25 mg/l 2, 4- D in the range 0.1-0.5 mg/l showed enhanced callus multiplication. Kn+ 2, 4-D at its 0.5 mg/l+ 0.25 mg/l concentration evoked best response. Kn proved to be a better choice than BAP. (Table-1, Figure-1 A, B, Figure-2). After 8 wk embryo was developed on MS medium supplemented with 0.5 mg/l BAP+ 0.25 mg/l 2,4-D and on MS medium supplemented with 0.5 mg/l Kn+ 0.25 mg/l 2,4-D (Figure-1 C,D). After development of embryos they are sub cultured to MS medium supplemented with BAP and Kn for shoot proliferation. Multiple shoot initiation were observed on medium containing 1 mg/l Kn. (Table-2, Figure-1 E, figure-3). Embryos also sub cultured on fresh medium containing IBA for root induction. Multiple rooting was observed on medium supplemented with 1mg/l IBA (Table-3, Figure-1 F, and Figure-4).

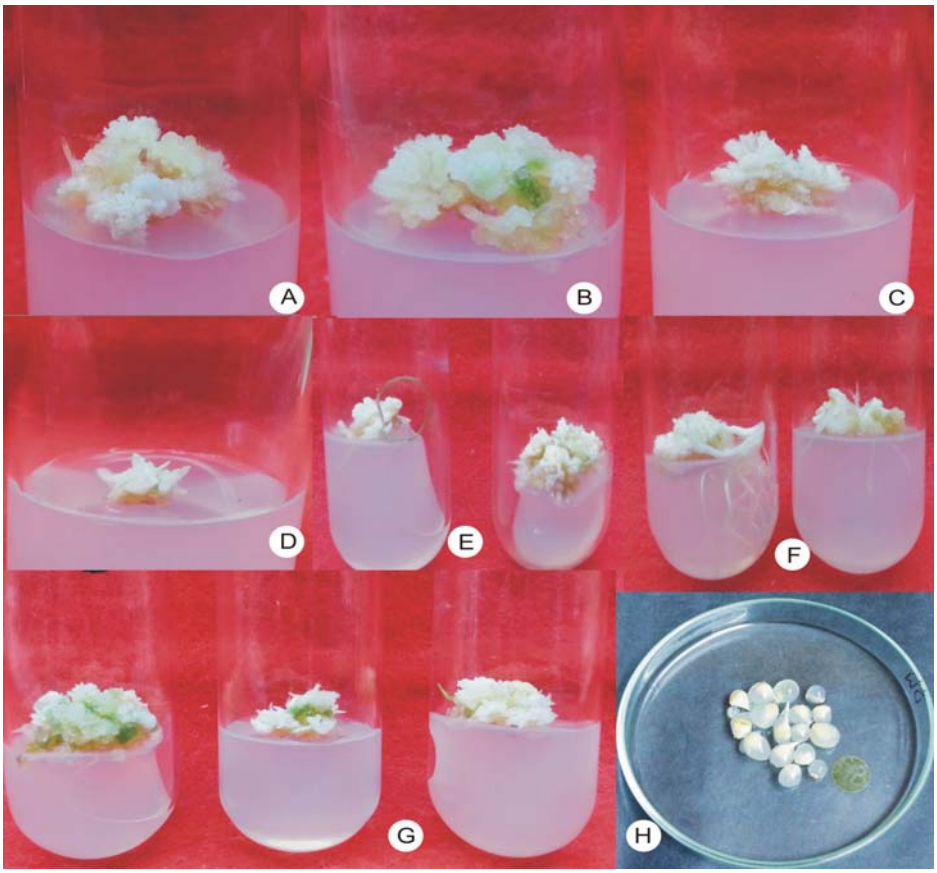
Fully mature somatic embryo of *Allium sativum* were used for the purpose of artificial seeds formation. Best seeds were prepared by using 3% sodium alginate and 1.1% $CaCl_2$. (Figure-1, H).

CONCLUSION

Micropropagated callus is used for the development of somatic embryo, as they are easy to establish in culture. In *Allium sativum* MS medium containing 0.5 mg/lKn + 0.25 mg/l 2, 4-D was the best for culture initiation. We have found that *Allium sativum* culture grew better on MS medium in comparison to other media. Ms medium supplemented with 0.5 mg/l Kn+ 0.25 mg/l 2, 4-D was most suitable for embryo development.

In *Allium sativum* 1.0 mg/l Kn was most suitable for shoot multiplication (Average number of shoots 3.42±0.39 and Average shoot length 7.54±0.31 cm).

IBA (Auxin) has been widely used as root induction hormone under *in vitro* and *in vivo* condition. We also found positive role of IBA during *in vitro* rooting. In *Allium sativum*, 1 mg/l IBA proved to be the best for in vitro rooting. Most responsive synthetic seed development was observed on MS medium with 3% sodium alginate and 1.1% $CaCl_2$ solution.



A. Callus multiplication on MS medium supplemented with 0.5 mg/l BAP+ 0.25 mg/l 2,4-D, B. Callus multiplication on MS medium supplemented with 0.5 mg/l Kn+ 0.25 mg/l 2,4-D, C. Embryo formation on MS medium supplemented with 0.5 mg/l BAP+ 0.25 mg/l 2,4-D, D. Embryo formation on MS medium supplemented with 0.5 mg/l Kn+ 0.25 mg/l 2,4-D, E. Multiple shoot formation on MS medium supplemented with 1 mg/l Kn. F. Multiple root formation on MS medium supplemented with 1 mg/l IBA, G. Multiple shoot proliferation on MS medium supplemented with 0.5 mg/l Kn+ 0.25 mg/l 2,4-D, H. Artificial seeds preparation with 3 % sodium alginate+ 1.1 % $CaCl_2$

Figure 1: (A-H) Micropropagation of *Allium sativum* from Callus multiplication

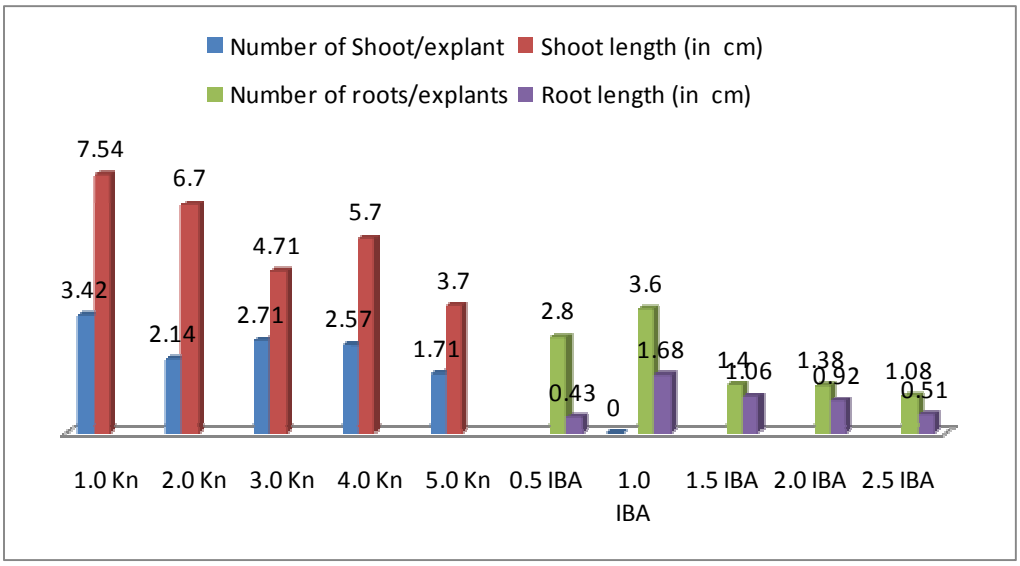


Figure 2: Effect of cytokine (Kn) & Auxin (IBA) on shoot and root induction from Callus of garlic

Table 1: Effect of different Hormones on Callus proliferation and Morphology

Hormone Conc. (mg/l)	Callus diameter after 7 weeks subculture (cm)	Callus proliferation Scoring	Color of callus	Morphology of callus
2,4-D + Kn				
0.25 + 0.3	2.6	++	Whitish	Friable
0.25 + 0.4	3.5	+++	Light green	Compact
0.25 + 0.5	4.6	++++	Whitish green	Nodular
2,4-D + BAP				
0.25 + 0.3	2.4	+++	Whitish	Friable
0.25 + 0.4	2.1	++	Whitish green	Trodden
0.25 + 0.5	3.4	++++	Whitish green	Nodular

'++++' Intense
'+++' Moderate
'++' Meager

Table 2: Effect of cytokine (Kn) on shoot proliferation by Callus of garlic

Hormone Concentration (mg/ l)	Number of Shoot/explant	Shoot length (in cm)	Shooting Response (%)
1.0 Kn	3.42±0.39	7.54±0.31	90
2.0 Kn	2.14±0.51	6.70±0.39	80
3.0 Kn	2.71±0.36	4.71±0.29	85
4.0 Kn	2.57±0.40	5.70±0.41	82
5.0 Kn	1.71±0.38	3.70±0.28	70

Medium: MS+ additives; mean± SD, n= 7 replicates. Means having the same letter in each Colum, do not different significantly at P< 0.05 (Tukey's test)

Table 3: Effect of Auxin (IBA) on root induction from isolated Callus of garlic

Hormone Concentration (mg/ l)	Number of roots/explants	Root length (in cm)	Rooting Response (%)
0.5 IBA	2.80±0.73	0.43±0.33	85
1.0 IBA	3.60±0.51	1.68±0.32	90
1.5 IBA	1.40±0.52	1.06±0.08	80
2.0 IBA	1.38±0.37	0.92±0.10	78
2.5 IBA	1.08±0.19	0.51±0.05	73

Medium: MS+ additives; mean± SD, n= 7 replicates. Means having the same letter in each Colum do not different significantly at P< 0.05 (Tukey's test)

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REFERENCES

[1] Butt et al.9; Iciek et al, 2009; Pittler and Ernst, 2007, Immunomodulation and reduction of cardiovascular disease risk factor. Environmental and molecular mutagenesis vol. 50, , (2009), no. 3, pp. 247-265.

[2] Kim MA, Park JK, 2002, High frequency plant regeneration of garlic (*Allium sativum* L.) calli immobilized in calcium alginate gel. Biotechnol. Bioprocess Eng. 7: 206-211.

[3] Tattelman , 2005, Health Effects of Garlic. *Am Fam Physician* 72(01): 103-106.

[4] Amagase H, 2006, Clarifying the real bioactive constituents of garlic. J. Nutr. 136: 716S-725S.

[5] MM Abo El-Nmmrganogenesis and embryogenesis in callus cultures of garlic (*Allium sativum* L., Plant Sci. Lett. (1977), 259-264.

[6] Al-Zahim MA, BV Ford-Lloyd, Newbury HJ, 1999, Detection of somaclonal variation in garlic (*Allium sativum* L.) using RAPD and +cytological analysis. Plant Cell Rep. 18: 473-477.

[7] Ayabe M, Sumi S, 1998, Establishment of a novel tissue culture method, stem-disc culture and its practical application to micropropagation of garlic (*Allium sativum* L.). Plant Cell Rep. 17: 773-779.

[8] X Barandiaran, N Martin, Rodriguez-Conde MF, Di Pietro A, Martin J 1999, An efficient method for callus culture and shoot regeneration of garlic (*Allium sativum* L.). HortSci. 34: 348-349.

[9] Bhagyalakshmi N, Thimmaraju R, Venkatachalam L, Murthy KNC, Sreedhar RV 2005. Nutraceutical applications of garlic and the intervention of biotechnology. Crit. Rev. Food Sci. Nutr. 45: 607 -621.

[10] Birch RG 1997. Plant transformation: problems and strategies for Lee et al. 1573 practical application. Ann. Rev. Plant Physiol. Plant Mol. Biol. 48: 297-326.

[11] Bockish T, Saranga Y, Altman 1997, A Garlic micropropagation by somatic embryogenesis. Acta Hort. (ISHS) 447, 241-242.

[12] Butt MS, Sultan MT, Butt MS, Iqbal J. 2009, Garlic: nature's protection against physiological threats. Crit. Rev. Food Sci. Nutr.49: 538-551.

[13] Martin-Urdiroz N, Garrido-Gala J, Martin J, Barandiaran X, 2004. Effect of light on the organogenic ability of garlic roots using a one-step *in vitro* system. Plant Cell Reports 22: 721-724.

[14] Bhojwani SS 1980, In *vitro* propagation of garlic by shoot proliferation. Scientia Hort. 13: 47 - 52.

[15] Bhojwani SS, Choen D and Fry PR, 1982, Production of virus free garlic and field performance of micropropagated plants. Scientia Hort. 18: 39 – 43.

[16] Stone OM, 1960, Factors affecting the growth of carnation plants from shoot apices. Ann. Appl. Biol. 52: 199-209.

[17] Vine SJ. 1960. Improved culture of apical tissues for production of virus free strawberries. J. Hort. Sci. 43:, 293 - 297.

[18] Walkey DGA, Webb JW, Bolland CJ and Miller 1987, A, Production of virus-free garlic (*Allium sativum* L.) an (1968d shallot (*A. ascalonicum* L.) by meristem-tip culture. J. Hort.Sci. 62: 211 - 220.