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Effect of Explant and Media Factors on Direct Shoot Organogenesis in Garlic (*Allium sativum* L.) cv. Ooty 2

Meena, S. [1]***, Gnanam, R.*** [2]***, Kavitha, M.*** [3]***, Radhamani, T.*** [4]***, Karthikeyan, M.*** [5] ***and Rajesh, S.*** [6*]

[1]*M.Sc. Scholar, Department of Plant Biotechnology,*
Tamil Nadu Agricultural University, Coimbatore, (Tamil Nadu) India.
[2]*Professor and Head, Department of Plant Molecular Biology and Bioinformatic,*
Tamil Nadu Agricultural University, Coimbatore, (Tamil Nadu) India.
[3]*Assistant Professor, Horticultural College and Research Institute,*
Tamil Nadu Agricultural University, Coimbatore, (Tamil Nadu) India.
[4]*Teaching Assistant, Department of Plant Biotechnology,*
Tamil Nadu Agricultural University, Coimbatore, (Tamil Nadu) India.
[5]*Assistant Processor, Horticultural College and Research Institute,*
Tamil Nadu Agricultural University, Coimbatore, (Tamil Nadu) India.
[6]*Assistant Processor, Department of Plant Biotechnology,*
Tamil Nadu Agricultural University, Coimbatore, (Tamil Nadu) India.

(Corresponding author: Rajesh, S.)*
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ABSTRACT: Garlic (*Allium sativum* L.) is one of the oldest cultivated crops known for its medicinal values and important spice used in culinary. In garlic, cloves are widely used as seed material for planting which poses challenges in increasing the input cost of cultivation and also there is questionable availability of quality planting material for the farmers. The present study was conducted to optimize the choice of explant for the direct organogenesis in the garlic cultivar Ooty 2. Direct organogenesis in garlic promotes micropropagation for virus-free bulblet production which could be employed as seed material. Shoot tip and basal meristematic region explants from garlic cloves were studied for their *in vitro* responses to shoot organogenesis. Basal region explants produced the maximum number of shoots in different shoot induction media compared to shoot tips. Mean number of shoots produced per clove is 3.2 when cultured in MS media supplemented with 0.1 mg L^{-1} NAA and 1 mg L^{-1} BAP. This is perhaps the first report on the direct shoot organogenesis in garlic cv. Ooty 2 and has important practical application as a source of secondary explant that can be used for production of *in vitro* bulblets that can be used as seed material by the garlic farmers.

Keywords: Garlic (*Allium sativum* L.), shoot tip, basal meristematic region, direct organogenesis.

INTRODUCTION

Garlic (*Allium sativum* L.) belongs to the genus Allium of the family Alliaceae which is the most diversified genus with nearly 114 species. It is a monocotyledonous diploid plant (2n =16) that has its origins in central Asia (Shemesh and Kamenetsky, 2021) and has been under cultivation for nearly 5000 years in India (Lawande *et al.,* 2009).

Garlic is a high value crop known for its therapeutic actions such as antimicrobial, anti-inflammatory, antidiabetic, chemotherapeutic, hepatoprotective and wound healing. These medicinal values are owing to the presence of a large number of organosulphur compounds like alliin, allicin, E-Ajoene, Z- Ajoene, S-allylcysteine and Allyl methyl sulphide (De Greef *et al.,* 2020). Garlic is also rich in enzymes, minerals, vitamins, fibre, amino acids and essential oils (El-Saber Batiha *et al.,* 2020). It holds a long history of being utilized in traditional medicine in many parts of the world (Singh and Singh, 2019).

Early reports on garlic micropropagation in various cultivars employed different techniques, in particular, root tip culture (Haque *et al.,* 1998; Hassan *et al.,* 2014), meristem culture (El-Nil 1977; Murkute and Gawande, 2018), thermotherapy combined with meristem culture (Manjunathagowda *et al*., 2021), twin-scale (Seabrook, 1994), shoot apex (Mohamed-Yasseen 1994; Gad El-Hak *et al*., 2011), stem-discs (Ayabe and Sumi 1998; Ayabe and Sumi, 2001; Xu *et al*., 2008), callus culture (Kim *et al.,* 2003; Mostafa *et al*., 2020) and inflorescence (Fan *et al*., 2017; Wen *et al*., 2020). Preference of the explant material is crucial for successful micropropagation since, the response of explants greatly vary depending on its size, quality and genotype in addition to the hormonal supplement (Smith, 2012). Culture responses like direct or indirect organogenesis also alter with the choice of explants. With this evidence, the present study was conducted to study the direct shoot organogenesis in the garlic cultivar Ooty 2 to optimize the choice explant and best media combinations for shoot regeneration in Garlic.

MATERIALS AND METHODS

Plant material. Garlic cultivar Ooty 2 bulbs were collected from the Tamil Nadu Agricultural University-Horticultural and Forestry Research Station, Udhagamandalam, Tamil Nadu. To reduce the microbial contamination, shade drying of garlic bulbs was done initially and stored for future *in vitro* culture use.

Explants preparation for shoot induction. Garlic bulbs were subjected to cold treatment at 4°C for four weeks before explant preparation. Cloves were separated from the garlic bulbs and outer protective dry leaves were peeled. Then they were pre-sterilized in distilled water 4-5 times, surface sterilized with 3-4 drops of Tween 20 in distilled water for 20 min with intermittent shaking and washed in distilled water for 4-5 times, then treated in 0.2 % (w/v) bavistin (Carbendazim 50 % WP) for 20 min and rewashed in distilled water for 4-5 times. Under Laminar airflow (LAF) condition, sterilization of cloves was done using 70 % (v/v) ethanol for 1 min followed by 3 % (v/v) NaOCl (Sodium hypochlorite) for 15 min and finally rinsed thoroughly with sterile distilled water for 4-5 times.

Shoot tip explants. Sterilized cloves were cut horizontally near the centre and outer storage leaves were removed. Shoot tip attached with the basal portion which is present inner to the storage leaves was excised along with the primordial leaves (Fig. 1a.) which were then split into four and inoculated in different shoot induction media such as MS basal (Murashige and Skoog, 1962) and MS media fortified with combinations of 1-Naphthaleneaceticacid (NAA) and 6-Benzylaminopurine (BAP) as mentioned in Table 1.

Table 1: Response of shoot tip explants on shoot organogenesis under different media combinations.

Treatment	Media Combination		Mean number of shoots/clove (Mean ±SE)*
	NAA (mg L^{-1})	**BAP** (mg L^{-1})	
T1	0	0	0.7(1.05±0.10)[az]
T2	0.1	1	0.7(1.07±0.08)[a]
T3	0.5	1	0.6(1.02±0.08)[a]
T4	1	1	0.6(1.02±0.08)[a]
T5	0.18 (1 µM)	2.25 (10 µM)	0.7(1.07±0.08)[a]

*CV= 25.67; Treatments found to be not significant at 5% level of significance
Means were compared by Duncan's Multiple Range Test; Treatments having the same letter are on par
SE=standard Error; z-square root transformed values

Basal region explants. The basal portion of the sterilized cloves (~1 cm) were cut and dissected into four parts which were then inoculated into jam bottles containing different shoot induction media as follows: Basal MS (Murashige and Skoog, 1962), Basal LS (Linsmaier and Skoog, 1965) and MS media fortified with 0.1 mg L^{-1} NAA and 0.1 mg L^{-1}, 0.5 mg L^{-1} or 1 mg L^{-1} of BAP.

All inoculations were carried out in the laminar air flow chamber. The pH of the media was adjusted to 5.8 ± 0.2 and the solid media was gelled with 0.8% (w/v) tissue culture grade agar and sterilized at 121°C for 20 min. prior to inoculation.

Culture maintenance. All the inoculated cultures were maintained in the primary growth room at 25±2° C under a light intensity of 1500 lux and photoperiod of 16/8 hours of light and dark. The number of shoots induced from both the explants was periodically recorded.

Statistical analysis. The experiment was done in a completely randomized design with ten and fifteen observations for shoot tip and basal region explants respectively. The data were analysed by one way ANOVA using WASP 2.0 (Web Agri Stat Package) and means were compared by Duncan's Multiple Range Test in OPSTAT online software (Sheoran, *et al.*, 1998).

RESULTS AND DISCUSSION

A. Choice of explant on direct organogenesis

Shoot tip: In this research, shoot induction of garlic from two explants *viz*., shoot tip and basal meristematic region were studied under different media combinations. The number of shoots regenerated from the shoot tip explants are relatively low with maximum of two shoots formed per clove (Table 1). Explant and the shoots formed were represented in Fig. 1(a, b). Shoots were differentiated after twenty days from the explants.

Mohamed-Yasseen *et al.*, (1994) reported 8.2 mean number of shoots from the intact and sliced shoot tip of garlic. Contrarily, in shoot apex culture of Tuniceli garlic, 1 to 2 shoots per explant was produced under low concentration of plant growth regulators whereas, the root tip culture was less effective for shoot production (Yanmaz *et al.*, 2010).

Basal region explant: The mean number of shoots per clove was higher (3.2) in basal region explants when compared to shoot tips. In all the media, shoot initiation started in 10-14 days of explant inoculation which was earlier than the shoot tip differentiation. This shows that basal region explants were found to be efficient than the shoot tips for direct shoot regeneration. Shoot induction from the basal region explants was depicted in Fig. 1(c, d). Root formation was observed concurrently with the initiation of shoots, but when shoot tip explants were used root initiation was infrequent.

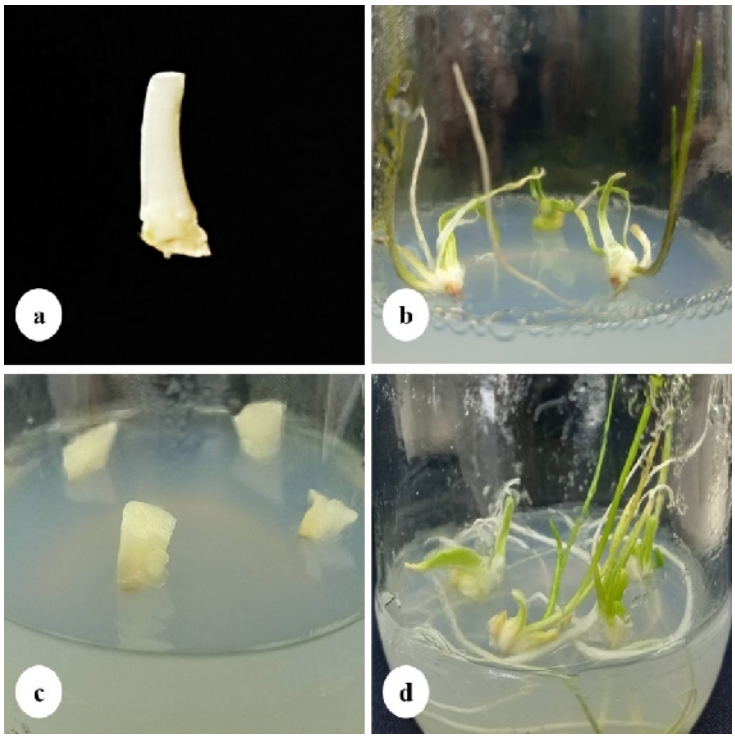

Fig. 1. Direct shoot organogenesis in Garlic cv. Ooty2 (a) Shoot tip along with leaf primordia (b) Regenerated shoots from shoot tip explant (c) Basal region explant after inoculation (d) Regenerated shoots from the basal region

B. Effect of plant growth regulators on shoot organogenesis

In this study, the media combinations used does not have any significant influence on the shoot induction from shoot tip explants. Similarly, when using shoot meristem along with leaf primordia as explants, MS medium supplemented with plant growth regulators were not significant from the basal medium and shoot differentiation was observed earlier in MS basal medium. Shoot growth was also inhibited by NAA at higher doses (Haque *et al.*, 2003).

MS medium supplemented with 0.1 mg L^{-1} NAA and 1 mg L^{-1} BAP (Table 2) responded the best for basal region explants with the maximum mean number of 3.2 shoots when compared to the other media however, the media combinations having 0.1 mg L^{-1} BAP, 0.5 mg L^{-1} BAP and 1 mg L^{-1} BAP were statistically similar.

NAA at higher concentrations results in the callusing of explants that might not be beneficial for direct organogenesis. So, a lower concentration of 0.1 mg L^{-1} of NAA was used. This concentration was found to be efficient for shoot growth in previous studies (Murkute and Gawande, 2018; Fan *et al.*, 2017; Seabrook, 1994). Media combinations without NAA or NAA combined with kinetin (kin) performed best for adventitious bud induction from inflorescence (Fan *et al.*, 2017). When high concentrations of BAP were used with a low concentration of NAA, hyperhydricity of shoots was observed. Those shoots were deformed with thick translucent leaves. Kim *et al.*, (2004) reported that removal of leaves was a prerequisite to prevent the hyperhydricity of shoots. Nagakubo *et al.*, (1993) observed that the higher KNO_3/NH_4Cl ratio in media reduced the vitrification of shoots along with multiple shoot induction. Gad El-Hak *et al.*, (2011) reported that low concentrations of plant growth regulators were suited for garlic plantlet production. Ayabe and Sumi (1998) observed that the response of shooting from the basal discs was restrained with the addition of higher doses of NAA or BAP.

Table 2: Response of basal region explants on shoot organogenesis under different media combinations.

Treatment	Media Combination			Mean Number of shoots/ clove (Mean ±SE)*
		NAA (mg L^{-1})	**BAP** (mg L^{-1})	
T1	Basal MS	0	0	1.93 (1.52±0.09)^{cz}
T2	Basal LS	0	0	2.40 (1.66±0.1)^{bc}
T3	MS	0.1	0.1	3.07 (1.88±0.22)^{ab}
T4		0.1	0.5	2.80 (1.79±0.08)^{ab}
T5		0.1	1.0	3.20 (1.92±0.05)^{a}

*CV= 16.678; Treatments found significance at 1% and 5% level of significance
CD (0.01) = 0.283 CD (0.05) =0.213
Means were compared by Duncan's Multiple Range Test; Treatments having the same letter are on par
SE=standard Error; z-square root transformed values

CONCLUSION

Explant and media optimization for the *in vitro* direct shoot regeneration in garlic cultivar Ooty 2 was developed in this study. The explants showed differential responses for the shoot regeneration. The basal region explant of the garlic was found to be the efficient explant than the shoot tips. This could also be excised more easily than the shoot tips and adopted when exploited on large scale. This report on micropropagation study for direct shoot organogenesis support and advance further researches on garlic, as a source of secondary explants for *in vitro* bulblet induction and genetic transformation procedures. Since bulblet induction demands quality shoots on larger scale, these findings from the present study in a popular commercial cultivar like Ooty2 has significance for future research on microbulblets production that can serve as quality planting material for the farmers.

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Conflict of interest: The authors declare no conflict of interest.

REFERENCES

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 Reports*, *17*(10): 773-779.

Ayabe, M., & Sumi, S. (2001). A novel and efficient tissue culture method– "stem-disc dome culture"–for producing virus-free garlic (*Allium sativum* L.). *Plant Cell Reports*, *20*(6): 503-507.

De Greef, D., Barton, E. M., Sandberg, E. N., Croley, C. R., Pumarol, J., Wong, T. L., & Bishayee, A. (2020). Anticancer potential of garlic and its bioactive constituents: A systematic and comprehensive review. In *Seminars in Cancer Biology*. *Academic Press*, *73*: 219-264.

El-Nil, M. M. A. (1977). Organogenesis and embryogenesis in callus cultures of garlic (*Allium sativum* L.). *Plant Science Letters*, *9*(3): 259-264.

El-Saber Batiha, G., Magdy Beshbishy, A., G Wasef, L., Elewa, Y. H., A Al-Sagan, A., El-Hack, A., & Prasad Devkota, H. (2020). Chemical constituents and pharmacological activities of garlic (*Allium sativum* L.): A review. *Nutrients*, *12*(3): 872.

Fan, B., He, R., Shang, Y., Xu, L., Wang, N., Gao, H., & Wang, Z. (2017). System construction of virus-free and rapid-propagation technology of Baodi garlic (*Allium sativum* L.). *Scientia Horticulturae*, *225*: 498-504.

Gad El-Hak, S. E. N. H., Ahmed, K. Z., Moustafa, Y. M., & Ezzat, A. S. (2011). Growth and cytogenetical properties of micro-propagated and successfully acclimatized garlic (*Allium sativum* L.) clones with a modified shoot tip culture protocol. *Journal of Horticultural Science & Ornamental Plants*, *3*(2): 115-129.

Haque, M., Wada, T., & Hattori, K. (1998). Garlic roots for micropropagation through in vitro bulblet formation. In: *XXV International Horticultural Congress, Part 10: Application of Biotechnology and Molecular Biology and Breeding-In Vitro*, 2000, 520: 45-52.

Haque, M. S., Wada, T., & Hattori, K. (2003). Shoot regeneration and bulblet formation from shoot and root meristem of garlic cv Bangladesh local. *Asian J. Plant Sci.*, *2*(1): 23-27.

Hassan, M. N., Haque, M. S., & Hassan, M. M. (2014). An efficient protocol for somatic embryogenesis of garlic *(Allium sativum* L.) using root tip as explant. *Journal of the Bangladesh Agricultural University*, *12*(452-2016-35615): 1-6.

Kim, E. K., Hahn, E. J., & Paek, K. Y. (2004). Morphological Development and Histology of Multiple Shoots and Microbulbs of Garlic Cultured in Bioreactors. *Journal of Plant Biotechnology*, *31*(4): 301-306.

Kim, E. K., Hahn, E. J., Murthy, H. N., & Paek, K. Y. (2003). High frequency of shoot multiplication and bulblet formation of garlic in liquid cultures. *Plant Cell, Tissue and Organ Culture*, *73*(3): 231-236.

Lawande, K. E., Khar, A., Mahajan, V., Srinivas, P. S., Sankar, V., & Singh, R. P. (2009). Onion and garlic research in India. *Journal of Horticultural Science*, *4*(2): 91-119.

Linsmaier, E. M., & Skoog, F. (1965). Organic growth factor requirements of tobacco tissue cultures. *Physiol. plant*, *18*(1): 100-127.

Manjunathagowda, D. C., Jayaswall, K., Singh, M., Sagar, R., & Chaturvedi, P. (2021). Thermotherapy of cloves for *in-vitro* mericlone production for healthy and sustainable management of garlic germplasm. *Indian Journal of Traditional Knowledge (IJTK)*, *20*(1): 262-266.

Mohamed-Yasseen, Y., Splittstoesser, W. E., & Litz, R. E. (1994). In vitro shoot proliferation and production of sets from garlic and shallot. *Plant cell, tissue and organ culture*, *36*(2): 243-247.

Mostafa, H. H., Wang, H., Song, J., & Li, X. (2020). Effects of genotypes and explants on garlic callus production and endogenous hormones. *Scientific reports*, *10*(1): 1-11.

Murkute, A. A., & Gawande, S. J. (2018). Production of virus free planting material through meristem culture in short day garlic cultivars Bhima Omkar and Bhima Purple. *Journal of Environmental Biology*, *39*(3): 286-290.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum*, *15*(3): 73-497.

Nagakubo, T., Nagasawa, A., & Ohkawa, H. (1993). Micropropagation of garlic through *in vitro* bulblet formation. *Plant Cell, Tissue and Organ Culture*, *32*(2): 175-183.

Smith, R. H. (2012). Explant preparation. Plant tissue culture: techniques and experiments. Academic Press. p.45-51.

Seabrook, J. E. (1994). *In vitro* propagation and bulb formation of garlic. *Canadian journal of plant science*, *74*(1): 155-158.

Shemesh, E., & Kamenetsky, R. (2021). Traditional and Novel Approaches in Garlic (*Allium sativum* L.). Breeding Advances in Plant Breeding Strategies: Vegetable Crops: Volume 8: Bulbs, Roots and Tubers. (Eds. Al-Khayri., J.M., Jain., M.S., and Johnson., V.D.) Bulb I, Springer Nature Switzerland AG, p. 3-50.

Sheoran, O. P., Tonk, D. S., Kaushik, L. S., Hasija, R. C., & Pannu, R. S. (1998). Statistical Software Package for Agricultural Research Workers. Recent Advances in information theory, Statistics & Computer Applications by D.S. Hooda & R.C. Hasija Department of Mathematics Statistics, CCS HAU, Hisar. 139-143.

Singh, R., & Singh, K. (2019). Garlic: A spice with wide medicinal actions. *Journal of Pharmacognosy and Phytochemistry*, *8*(1): 1349-1355.

Wen, Y. B., Liu, X. X., Liu, H. J., Wu, C. N., Meng, H. W., & Cheng, Z. H. (2020). High-frequency direct shoot organogenesis from garlic (*Allium sativum* L.) inflorescence and clonal fidelity assessment in regenerants. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *141*(2): 275-287.

Xu, Z., Um, Y. C., Kim, C. H., Lu, G., Guo, D. P., Liu, H. L., & Mao, A. (2008). Effect of plant growth regulators, temperature and sucrose on shoot proliferation from the stem disc of Chinese jiaotou (*Allium chinense*) and in vitro bulblet formation. *Acta Physiologiae Plantarum*, *30*(4): 521-528.

Yanmaz, R., Yazar, E., Kantoglu, K. Y., & Alper, A. (2010). *In vitro* plant regeneration and bulblet formation of Tunceli garlic (*Allium tuncelianum* (Kollman) Özhatay, Matthew, Siraneci) by shoot and root culture. *Journal of Food, Agriculture & Environment*, *8*(3-4): 572-576.

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