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In vitro shoot organogenesis in therapeutically important spice crop, Kodaikanal hill garlic (*Allium sativum* L.)

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Abstract
Garlic is a one of the nature's versatile medicinal plant, having importance in culinary purpose. It is vegetatively propagated through cloves but due to its impotency to produce potent seeds and more infection with complex of viruses leads to yield loss. Quality seed material is very important for good production, farmers are facing huge loss due to scantiness in supply of good quality seed material. The current study was performed to optimize the media for direct shoot organogenesis in garlic genotype Kodaikanal hill garlic. Micropropagation of garlic through direct organogenesis aims to produce virus free planting material. Garlic clove basal meristematic regions were studied for their *in vitro* responses in shoot organogenesis. The number of shoots produced through basal meristematic region explant in different shoot induction media was analyzed. MS media supplemented with 2 mg L^{-1} BAP and 0.1 mg L^{-1} NAA produced an average 3.1 shoots per clove. These findings of the study will be useful for scaling up the protocol for shoot organogenesis and subsequent bulblet induction in garlic.

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

Introduction
Garlic (*Allium sativum* L.) is an ancient domesticated spice which belongs to the genus Allium, and it is the most diverse genus in the family Alliaceae, with roughly 114 species. It is a diploid (2n =16) plant belonging to monocotyledons and is vegetatively propagated through cloves (Shemesh and Kamenetsky, 2021) [1]. Garlic was having low reproduction coefficients because of its long term asexual propagation (Fan *et al.*, 2022) [2]. It is preferably quoted as a medicinal plant because of its therapeutic properties like anti-bacterial, antifungal, anti-protozoal, antiviral, antioxidant, anti-inflammatory, immuno-modulatory activity, anti- SARS-CoV-2 and most importantly in culinary purposes. Garlic clove contains 65% water, 28% carbohydrates, 1.5% fiber, 1.2% free amino-acids, 2.3% organosulphur compounds and 2% proteins. The medicinal and aromatic properties of garlic is due to organosulphur compounds mainly allicin (Moutia *et al*., 2018); Mosbauer *et al*., 2021) [3, 4]. Garlic is grown in almost all climatic conditions like temperate to sub–tropical climate and is cultivated in whole world with total production of 2,80,54318 Tonnes in the world. India is second highest garlic production country which is about 29,17000 Tonnes in 2020 (FAO 2021).
In Tamil Nadu, garlic is mainly cultivated in Nilgiris and Kodaikanal hill regions. Kodaikanal hill garlic is having special demand and it has been given geographical indication tag by Govt. of India during 2019-2020 for its novelty [5].
This is perhaps first report on *in vitro* micropropagation on direct shoot organogenesis in Kodaikanal hill garlic and this has a greater scope in producing microbulblets subsequently for use as seed material by the farmers.

Materials and Methods
Plant material processing
Kodaikanal hill garlic was procured from the leading garlic growers in Poomparai areas of Kodaikanal. The cloves were separated and bulbs were shade dried to minimize the microbial contamination and stored in cloth bag for further *in vitro* culturing experiment.

Explants preparation for shoot induction
Before explant preparation, garlic bulbs were exposed to a four-week cold treatment at 4 °C.

Outer dry layer of cloves were removed. Pre-sterilization was done by peeling off garlic cloves, washing with distilled water and treatment with 3-4 drops of Tween-20 for 18-20 min. with intermittent shaking. Cloves were washed with distilled water and further treated with 0.8% (w/v) bavistin for 20 min. and rinsed with sterile water for 4-5 times. After bavistin treatment, explants were taken to laminar air flow condition and sterilized with 70% ethanol for 45 sec. followed by 2.5% (v/v) NaOCl (Sodium hypochlorite) for 12 min. The explants were washed with sterile distilled water for 3-4 times and blot dried on sterile tissue paper.

Inoculation and culture maintenance

Under sterile conditions, the sterilized cloves' basal region (1 cm) was cut and split into four portions, which were subsequently inoculated into jam bottles containing shoot induction medium with combinations of growth hormones *viz.,* MS basal media fortified with 0.1 mg L^{-1} NAA and 0.5 mg L^{-1}, 1 mg L^{-1}, 1.5 mg L^{-1}, 2 mg L^{-1} BAP (Murashige and Skoog, 1962) [6]. The pH of the medium was adjusted to 5.8 ± 0.2, and tissue culture grade agar was added as gelling agent with 0.8% (w/v) and sterilised for 20 min. at 121°C using autoclave.

All inoculated cultures were incubated at 25 ± 2 °C with a light intensity of 1500 lux and photoperiod of 16/8 hours of white light and dark in the primary growth chamber. The number of shoots induced from both explants were taken into consideration on a regular basis.

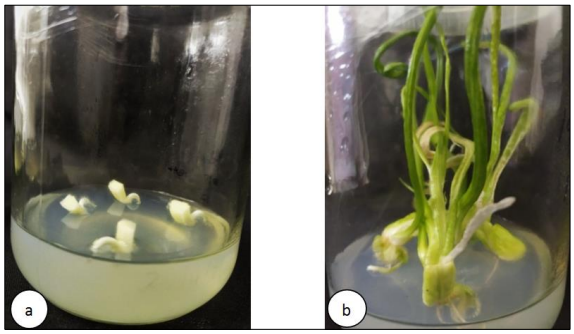
Statistical analysis

The experiment was done in a completely randomized design with replicates for each media combinations. The data were analyzed 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) [7].

Results and Discussion

Effect of plant growth regulators on shoot organogenesis

In shoot cultures of Elephant garlic, Seabrook (1994) [8] found that MS medium supplemented with 0.5 M NAA and 2 mg L-1 BAP was optimum for shoot initiation and subsequent axillary shoot multiplication from the basal plate explants (*Allium ampeloprasum*). In our study, the mean number of shoots per clove generated was maximum of 3.1 in the treatment, where MS media supplemented with 0.1 mg L^{-1} NAA and 2 mg L^{-1}BAP (Table 1). Shoot initiation began 10-14 days after explant inoculation in all the media combinations. For direct shoot regeneration, basal region explants showed positive results and has been successfully established from our earlier findings (unpublished). Fig. 1 (a, b) shows shoot induction in explants from the basal area. Root development occurred simultaneously with the initiation of shoots.



a) Basal region explant after inoculation b) Regenerated shoots from the basal region ready for bulblet induction

Fig 1: Direct shoot organogenesis in Kodaikanal Hill Garlic

According to Meena *et al.* (2021) [9], the basal region explants of the garlic cultivar Ooty2, produced the highest mean number of 3.2 shoots in MS media supplemented with 0.1 mg L^{-1} NAA and 1 mg L-1 BAP. NAA at higher concentration induce callus so it is not beneficial in direct organogenesis but lower concentration showed good response which is corroborated with the findings of Seabrook (1994) [8]; Fan *et al.* (2017) [10]; Murkute and Gawande (2018) [11]. Contrastingly Ayabe and Sumi (1998) [12] reported, retardation of shoot formation from the basal disc regions at higher concentration of BAP or NAA. Our study showed positive influence of shoot induction with additional supplementation of BAP at higher concentration (2.0 mg L^{-1}BAP). This improved response to shooting with cytokinin is agreeable with the reports of Hajare *et al.* (2021) [13] in potato micropropagation, with two varieties, Belete and Gudiene producing more number of shoots in MS media supplemented with 1.0 mg L^{-1} BAP and 2.0 mg L^{-1} NAA and 1.5 mg L^{-1}BAP + 3.0 mg L^{-1}NAA respectively. Htwe *et al.* (2021) [14] studied *in vitro* shoot regeneration through indirect organogenesis in Lily and have reported highest shoot regeneration in cultures of MS media supplemented with 2.5 mg L^{-1} BAP and 2.5 mg L^{-1} NAA.

In our study some plants showed hyperhydricity, it is a physiological disorder of *in vitro* plants observed when media supplemented with increased cytokinin and lower level of NAA concentration. Earlier reports indicate, loss of cultures due to hyperhydricity amounting to 60% in Micropropagation, where shoots appear glassy and wrinkled shape when media is supplemented with BAP (Wu *et al.*, 2008) [15]. Liu *et al.* (2017) [16] reported hyperhydricity with increased concentration of cytokinin and this is related to our observations in high levels of BAP causing hyperhydricity of *in vitro* shoots.

Table 1: Shoot organogenesis in garlic under different media combinations through

Treatment	Media	Hormone Combination		Mean Number of shoots/clove (± SD)
		NAA (mg L^{-1})	BAP (mg L^{-1})	
T1	MS	0	0	2.4^{b} (± 0.995)
T2		0.10	0.50	1.65^{c} (± 0.745)
T3		0.10	1.00	2.05^{b} (± 0.759)
T4		0.10	1.50	2.1^{bc} (± 0.911)
T5		0.10	2.00	3.1^{a} (± 0.852)

Data represented as mean ± SD, Coefficient of Variation = 37.955
Treatments found Significant at 1% and 5% level of significance
CD (0.01) = 0.713 CD(0.05) = 0.538
Means were compared by Duncan's Multiple Range Test; Treatments having the same letter are on par.

Conclusion

In this study, attempts were made to optimize media conditions for direct shoot organogenesis in Kodaikanal hill garlic using the basal part of garlic clove as explant. The shoots obtained can be successfully used as secondary explant for healthy micro bulblets production. The microbulblets can be used as seed material by farmers, which can make farmers to overcome problem of scarce supply of quality seed material for garlic cultivation.

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