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*Full Length Article*

# Standardization of Meristem Tip Culture in Short Day Garlic Varieties

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## Abstract

The garlic is one among the major crop in the genus *Allium* and mainly consumed as spice. It is asexually propagated through cloves, thus lead to the infestation with numerous viruses and were cause serious threat for its successful cultivation. Availability and assess to the virus free planting material is difficult, hence the experiment was carried out to standardize the protocol for meristem tip culture using MS medium along with different hormone combination to produce quality seeding cloves of garlic varieties namely G-41, G-282 and G-323. Results revealed maximum percentage survival of meristem in the treatment, where meristem was taken from fresh cloves. Overall, best combinations in case of meristems taken from sprouted cloves for initiation and multiplication of meristem tip in garlic was observed in Murashige and Skoog medium supplemented with thidiazuron (TDZ) (1.0 mg/L) with NAA (0.15 mg/L) and TDZ (1.0 mg/L) with NAA (0.05 mg/L). Hence, this protocol can be utilized for commercial meristem tip culture of garlic varieties for the production of virus free planting material. Standardized media is found suitable for the initiation and multiplication of meristem taken from old cloves as well as fresh clove. Virus free cloves can be directly used for initiation and multiplication, through meristem tip culture, whereas in case of virus infected cloves the meristems can be taken from the sprouted cloves, where the chances of getting virus free material is high. 

**Keywords:** Clove; Garlic; *In vitro* culture; Media combination; Meristem culture; Thidiazuron

## Introduction

Garlic (*Allium sativum.* L) is a vegetatively propagated crop, as a result sexual reproduction is absent, thus cloves are used for propagation. Hence, vegetatively propagated plants are systemically infected with diseases, the pathogens pass from one generation to the next through vegetative propagules. Thereby entire variety which is propagated by clonally may be infected with the pathogens, degenerates the planting material, in turn cause losses to bulb production and productivity. It is infected with a number of viral diseases and reported to cause 70% bulb yield loss (Nagakubo *et al.* 1991; Ayabe and Sumi 2001). The important viruses influencing yield and quality are garlic common latent carlavirus (GCLV), garlic dwarf reovirus, leek yellow stripe potyvirus (LYSV), mite-borne filamentous viruses (MbFV), onion yellow dwarf potyvirus (OYDV), shallot latent carlavirus (SLV) and serologically related carlaviruses, shallot yellow stripe potyvirus (SYSV), tomato spotted wilt virus (TSWV), carnation latent virus (CLV), leek yellow stripe virus (LYSV), garlic mosaic virus (GMV), garlic virus X (GVX), shallot virus X, narcissus latent virus, and iris yellow spot virus (Manjunathagowda *et al.* 2017; 2021). Symptoms of latent viruses are difficult to detect, many viruses may not even show visible symptoms, and pathogen attack does not always lead to death of plants, however the presence of viruses reduce the crop yield and quality of produce (Wang and Hu 1980). The viruses causing yield loss due to poty virus and carlaviruses, which ranged between 30–40% (Sing 2005). Elimination of viruses from planting materials is highly desirable to optimize the yield, and also to facilitate the hassle-free movement of living plant materials across the international boundaries (Manjunathagowda *et al.* 2017; 2021). It is difficulties to induce flowering in this species, thus possess problem for the breeder to breed promising genotypes, there is need to develop disease tolerant and resistant varieties against viral diseases, which is difficult task in garlic, due to breeding is limited to clonal selection. However, disease free planting material can be produced through meristem tip culture (Barandiaran *et al.* 1998). In

infected plants, apical meristems either free or carry a very low load of viruses (Quak 1977; Wang and Hu 1980). Thus pathogen-free propagules are further multiplied vegetatively for commercial production. However, isolation of shoot meristem, their production has been focused mainly during long day type of garlic cultivars, there is need to standardize technique for meristem tip culture protocol for shoot and root multiplication in short day garlic varieties, further virus free material can be used for multiplication in large quantity in virus free areas. Hence, the present study was undertaken to standardize a protocol for the micro propagation of short-day garlic through meristem tip culture.

## Materials and Methods

### Study materials

Three different genotypes of garlic namely G-41, G-282 and G-323 released by National Horticultural Research and Development Foundation, Nasik, India were used in the study.

### Preparation of explants and culture media

Preparation of mother plant to provide quality explant for better establishment of aseptic cultures. Cloves were used for raising *in vitro* meristem cultures; the experiment was sorted in two sets. In first set, the explant was taken directly from the fresh clove. In second set, explant are 15–20 old days sprouted cloves. Meristem tip culture of garlic was initiated using meristematic region from sprouted cloves, and fresh cloves (stored) which was excised and inoculated on treatments of culture media.

The MS medium powder procured from HiMedia, India, used for media preparation. Initially, container of 1 L volume was rinsed with the distilled water, washed thoroughly. Then, 800 mL of distilled water was taken in the container, along with MS medium powder, with suitable treatment combinations of plant growth hormones, with TDZ and NAA in different combinations (the concentration of stock solution was 1 mg/mL) along with control as MS medium. The media combinations presented in Table 1, followed by stirring the water till the medium was dissolved completely to a clear solution and then final volume was made to 1 L. Then, pH was adjusted to 5.77 to 5.82 by using 1 N NaOH and HCl. Gelling agent agar was added 8 g of (0.8%) per liter of media and it was boiled till the agar dissolve completely to obtain a clear solution. Dispensed the medium in suitable culture vessels (test tubes). These tubes containing medium were autoclaved at 15 lb/inch$^2$ pressure and 121$^o$C temperature for 15 min. The medium was allowed to cool and used for the inoculation.

### Sterilization, inoculation of explants and initiation of culture

The surface sterilization of explant (the outer dry layers removed of cloves) was done by rinsing the cloves for three times under running tap water, followed by rinsing with 70% alcohol for two min. Then cloves were treated with Bavistin (0.1%) along two drops of Tween-20 for 15 min. Then washed the cloves with water for one hour, to remove traces of Bavistin. Soon after, cloves were treated with 70% alcohol for two minutes in the laminar airflow and washed for thrice with sterile distilled water. Then the cloves were treated with sterilant NaClO (2% chlorine), Qualigens, India for 15 min, followed by washing with sterile distil water for three to four times, then finally the surface sterilized cloves were used for inoculated in the media.

The meristem tip was inoculated on 16 different media compositions of MS media (Murashige and Skoog 1962) with the addition of TDZ and NAA in 5 replications for initiation of the cultures. The meristematic tip was dissected from the cloves and inoculated on treatment media. The explants were inoculated on the medium under aseptic condition, the cultures were incubated at 25±2$^o$C under cool light of 3000 lux and 16:8 h light/dark regime for 15–20 days.

### Isolation and inoculation meristems, incubation meristems culture and their multiplication

Meristem tip were dissected from 15–20 days old, sprouted shoot tip, as well as from fresh clove under ascetic condition, with help of sterilized micro-scalpels in laminar air flow, using the forceps were used for isolation of meristem the microscope. Short hypodermic needles were used to remove leaves and their primordia. This technique was used to avoid transfer of the microorganisms to the sterile apex. A thin layer of sterile distilled water used to create contact in between explant and medium surface.

The dissected meristem was inoculated on 16 different culture media composition (Table 1) under aseptic condition and culture were incubated at 25±2$^o$C under cool light of 3000 lux and 16:8 h light/dark condition. Observations were made after 15 days and 1 month of inoculation in both sets of experiment. Plant height and number of shoots were recorded for five replications and data was analysed in completely randomized design. The multiple shoot formation led to the maximum number of propagules; however, in culture, a meristem may develop either into single shoot or multiple shoot masses or even into rooted plantlets.

### Preparation of media, inoculation, incubation and establishment of multiple shoots

The relatively high cytokinin concentrations in cultures enhance the axillary branching. The MS medium added with varied concentration of the BA (0.5, 1.0, 1.5, 2.0) and 2-ip (0.5, 1.0, 1.5, 2.0) was used, the plant growth regulators and their concentration presented in the Table 2. The apical portion of the single shoot was sliced by fine blade and inoculated on the media compositions under aseptic condition and culture were incubated at 25±2$^o$C under cool light of 3000

lux and 16:8 h light/dark regime for 1 month. The nutrient medium containing the less plant regulator concentration for vigorous growth was used. The media preparation was done with varied plant growth regulators and their concentration for establishment of cultures for the growth of small shoots and to increase the number of plants (Table 3). The multiple shoots were inoculated on the media compositions as shown in Table 3 and culture were incubated at 25±2ºC under cool light of 3000 lux and 16:8 h light/dark regime.

## Results

Murashige and Skoog (1962) medium was employed for the initiation of cultures supplemented with TDZ and NAA in 16 different media combinations in three garlic varieties. In garlic, initiation of meristem cultures taken from sprouted cloves started within 8–10 days, while it took 10 to 12 days from the meristems of fresh cloves. All the varieties showed similar response in all the combinations. The sprouted cloves are metabolically active; thus, it may show the early response in initiation of cultures and induced the early multiplication.

Shoot initiation, regeneration and multiplication was recorded in all the media combinations in the established meristem cultures. Auxins and cytokinins were induced the shoot growth individually, or in combinations, the shoot initiation from meristem was delayed by 2–3 days from fresh cloves as compared to meristem isolated from sprouted cloves. Average percentage of regeneration of meristem was 91.6% from sprouted cloves and 95% from fresh cloves. In case of meristem taken from sprouted cloves the average percentage of regeneration of meristem was 82.5, 96.8 and 90.6% in G-41, G-282 and G-323, respectively (Table 4). There was difference in height of shoot in different varieties. The few combinations showed 100% regeneration, elongation and multiplication of shoots among the varieties (Table 4; Fig. 1A–C). In G-41, TDZ at 1.0 mg/L aided for 100% regeneration, and all the combinations of TDZ and NAA showed good response and vigorous growth of garlic varieties.

Average percentage of regeneration of meristem was 95% in all the varieties, MS medium in addition of TDZ and NAA individually or in combination in all 16 media combinations was noted 80–100% regeneration rate. The height of meristem was about 5 to 10 mm within 15 days of inoculation in different treatment combinations among all varieties. The maximum shoot height of plants derived from sprouted cloves meristems was 9.5 mm recorded in variety G-41. MS medium supplemented with NAA gave 6.75 mm an average height but does not have vigorous growth and the leaves become pale yellow in color, cultures become poor and showed pale yellow coloration.

After one month, in the variety G-41, the shoot height was maximum (31.0 mm), which was statistically at par other treatments, in the meristem taken from sprouted cloves (Table 4). In variety G-282, the maximum shoot height was 24.75 mm and in the variety G-323 the shoot height of 27.5 mm was recorded and were statistically at par to other, treatment combinations (Table 4). In G-41, shoot height was increased with increase of individual concentration of TDZ. Combinations gives significantly highest shoot height in variety G-323 and showed vigorous growth was presented in Table 4. Shoot height at one month of inoculation of meristem taken from fresh clove was significantly highest in variety G-41 (33.75 mm), which was statistically at par to other combinations. In G-41 increasing the individual concentration of TDZ increases the height of shoots, but in combination with increasing concentration of NAA, decreased the growth of shoots.

Maximum number of shoots was recorded at one month of inoculation, through increasing concentration of TDZ and NAA, as well as the number of multiple shoots was increased. The average number of shoots per meristem from

**Table 1:** Media composition and amount of plant growth regulators added for initiation of cultures in garlic

| No. | Treatments [PGR (mg/L)] | TDZ (µ/L) | NAA (µ/L) |
|---|---|---|---|
| 1 | MS basal medium | - | - |
| 2 | MS basal medium + TDZ (0.1 mg/L) | 6 | - |
| 3 | MS basal medium + TDZ (0.5 mg/L) | 30 | - |
| 4 | MS basal medium + TDZ (1.0 mg/L) | 60 | - |
| 5 | MS basal medium + NAA (0.05 mg/L) | - | 3 |
| 6 | MS basal medium + NAA (0.1 mg/L) | - | 6 |
| 7 | MS basal medium + NAA (0.15 mg/L) | - | 9 |
| 8 | MS basal medium + TDZ (0.1 mg/L) + NAA (0.05 mg/L) | 6 | 3 |
| 9 | MS basal medium + TDZ (0.1 mg/L) + NAA (0.1 mg/L) | 6 | 6 |
| 10 | MS basal medium + TDZ (0.1 mg/L) + NAA (0.15 mg/L) | 6 | 9 |
| 11 | MS basal medium + TDZ (0.5 mg/L) + NAA (0.05 mg/L) | 30 | 3 |
| 12 | MS basal medium + TDZ (0.5 mg/L) + NAA (0.1 mg/L) | 30 | 6 |
| 13 | MS basal medium + TDZ (0.5 mg/L) + NAA (0.15 mg/L) | 30 | 9 |
| 14 | MS basal medium + TDZ (1.0 mg/L) + NAA (0.05 mg/L) | 60 | 3 |
| 15 | MS basal medium + TDZ (1.0 mg/L) + NAA (0.1 mg/L) | 60 | 6 |
| 16 | MS basal medium + TDZ (1.0 mg/L) + NAA (0.15 mg/L) | 60 | 9 |

**Table 2:** Media composition used for the multiplication of cultures

| No. | Treatments (mg/L) | Volume of PGR (µL) |
|---|---|---|
| 1 | MS basal medium | 1 |
| 2 | MS basal medium + BA (0.5 mg/L) | 50 |
| 3 | MS basal medium + BA (1.0 mg/L) | 100 |
| 4 | MS basal medium + BA (1.5 mg/L) | 150 |
| 5 | MS basal medium + BA (2.0 mg/L) | 200 |
| 6 | MS basal medium + 2-ip (0.5 mg/L) | 50 |
| 7 | MS basal medium + 2-ip (1.0 mg/L) | 100 |
| 8 | MS basal medium + 2-ip (1.5 mg/L) | 150 |
| 9 | MS basal medium + 2-ip (2.0 mg/L) | 200 |

**Table 3:** Media composition for the establishment of cultures and amount of PGR to be added

| No | Treatments (mg/L) | PGR | |
|---|---|---|---|
| | | TDZ (µL) | NAA (µL) |
| 1. | MS basal medium. | - | - |
| 2. | MS basal medium + TDZ (0.1 mg/L) | 300 | - |
| 3 | MS basal medium + TDZ (0.5 mg/L) | 500 | - |
| 4. | MS basal medium + TDZ (0.1 mg/L) + NAA (0.05 mg/L). | 300 | 15 |
| 5. | MS basal medium + TDZ (0.1 mg/L) + NAA (0.1 mg/L). | 300 | 30 |
| 6. | MS basal medium + TDZ (0.5 mg/L) + NAA (0.05 mg/L). | 1500 | 15 |

**Table 4:** The performance of meristem cultures of garlic varieties in different treatment combinations

| Treatment | G -41 | | | | G-282 | | | | G-323 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Regenerate meristem (%) | Shoot height (15 days) | Shoot height (1 month) | Shoots per meristem | Regenerate meristem (%) | Shoot height (15 days) | Shoot height (1 month) | Shoots per meristem | Regenerate meristem (%) | Shoot height (15 days) | Shoot height (1 month) | Shoots per meristem |
| 1 | 60 | 2.75 | 9.30 | 0.6 | 100 | 3.5 | 9.0 | 1.0 | 100 | 3.5 | 6.0 | 1.0 |
| 2 | 60 | 5.6 | 21.0 | 0.6 | 100 | 4.25 | 16.25 | 1.7 | 100 | 6.0 | 9.0 | 1.5 |
| 3 | 80 | 9.5 | 21.3 | 0.8 | 100 | 3.0 | 17.5 | 2.0 | 100 | 8.0 | 14.0 | 4.0 |
| 4 | 100 | 5.1 | 25.0 | 1.0 | 100 | 4.5 | 12.5 | 1.0 | 100 | 8.0 | 17.5 | 1.0 |
| 5 | 80 | 4.4 | 22.0 | 1.0 | 100 | 5.5 | 13.75 | 1.0 | 100 | 8.5 | 24.5 | 1.0 |
| 6 | 60 | 4.0 | 16.4 | 0.6 | 100 | 5.5 | 13.0 | 1.0 | 100 | 8.0 | 23.5 | 1.0 |
| 7 | 60 | 4.25 | 9.8 | 0.6 | 75 | 3.0 | 14.5 | 1.0 | 100 | 11.0 | 16.0 | 1.0 |
| 8 | 100 | 5.8 | 21.2 | 2.4 | 100 | 4.0 | 15.0 | 1.0 | 100 | 10.0 | 22.5 | 1.0 |
| 9 | 100 | 6.3 | 29.0 | 1.4 | 100 | 5.0 | 13.0 | 2.5 | 100 | 7.5 | 27.5 | 2.0 |
| 10 | 60 | 6.6 | 17.2 | 0.6 | 75 | 3.5 | 17.6 | 1.25 | 50 | 1.5 | 12.5 | 0.5 |
| 11 | 80 | 7.75 | 22.0 | 1.4 | 100 | 4.75 | 13.75 | 3.2 | 100 | 3.5 | 6.5 | 1.0 |
| 12 | 100 | 5.0 | 13.2 | 1.2 | 100 | 3.5 | 16.25 | 2.0 | 50 | 2.0 | 7.5 | 0.5 |
| 13 | 100 | 3.0 | 13.4 | 1.2 | 100 | 5.0 | 15.0 | 2.2 | 50 | 2.0 | 6.0 | 0.5 |
| 14 | 100 | 3.0 | 8.1 | 2.8 | 100 | 5.5 | 18.2 | 2.7 | 100 | 4.5 | 24.0 | 5.5 |
| 15 | 80 | 2.6 | 9.0 | 1.0 | 100 | 2.25 | 9.5 | 1.7 | 100 | 6.0 | 6.5 | 1.5 |
| 16 | 100 | 1.8 | 10.8 | 3.8 | 100 | 5.25 | 10.5 | 4.0 | 100 | 6.5 | 23.5 | 1.5 |

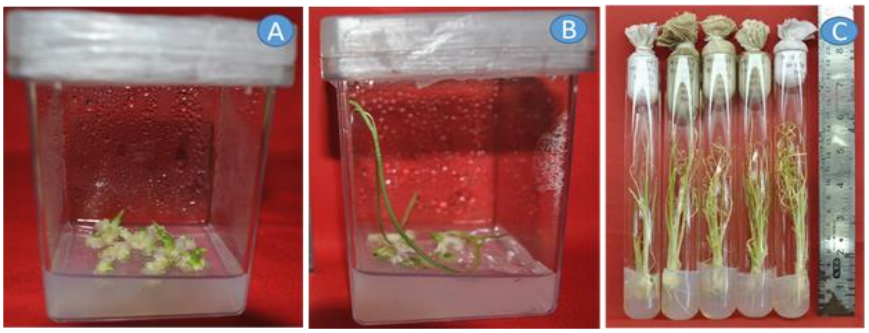


**Fig. 1:** A) The regeneration of meristems, B) Elongation of shoots from the meristems, and C) multiplication of plants derived from meristem culture

sprouted clove ranged from 1.16 in G-323 to 1.34 in G-282. In the variety G-41, maximum number of shoots and was 4.25 in the variety G-323, number of shoots was 1.75. Overall, it was recorded that, as the number of multiple shoots per meristem increased, it resulted in decreased height of shoots, with reduced growth of shoot. Initiation of number of shoots in meristem isolated from the fresh clove gave delayed response as compared with the meristem from the sprouted cloves. Variety G-282 gave better response with 1.23 average number of shoots as compared with G-41 (1.02) and G-323 (1.11). Average number of shoot initiation in variety G-41 was non-significant in all the combinations, whereas in variety G-282 the average number of shoots were maximum.

In meristem from sprouted clove good number of multiple and healthy shoots were obtained within one month, whereas it took about a month to initiate the multiple shoot formation in case of meristem taken from fresh cloves, which reduced multiplication in meristem taken from sprouted cloves. Where the number of shoots are more at later stage, mainly after one month, the growth of multiple shoots is affected. Hence, it is necessary to subculture the shooted plants to establishment medium for vigorous growth. Establishment media contain the low concentration of plant growth regulators, which support for the growth of small shoots, while nutrients available in the media lead to the vigorous growth of the shoots. After transferring it into the establishment media, within 15–20 days the plant grows vigorously.

## Discussion

All the combinations of TDZ and NAA showed good response and vigorous growth. But in less concentration of TDZ (0.1 mg/L) and high concentration of NAA (0.15 mg/L) the percentage of regeneration decreased to 60%. It was noticed that high concentration of auxin suppressed the percentage of shoot regeneration (G-41). But in the variety G-282 and G-323, low concentration of TDZ also induced the shoot regeneration. The auxin induced the shoot regeneration, when applied individually; gradually leaves become pale yellow in color, while the vigorous growth of plants in the combinations having auxins and cytokinin may be due to accumulation of the chlorophyll which promoted the conversion of etioplast into chloroplast. The delayed shoot initiation of meristems may be due to the dormant stage of meristem in fresh cloves and took 2 to 3 days to initiate the cell division. In G-41, increasing the individual

concentration of TDZ increases the height of shoots, but in combination, with increasing concentration of NAA, decreased the growth of shoots. The high concentration of NAA reduces the growth of shoots and leaves become pale yellow in color which was also supported by the findings given by (Haque *et al*. 1997). Average number of meristems was comparatively more from sprouted cloves than the meristem taken from fresh cloves. The meristem in fresh clove may be in dormant stage as compared with that of sprouted cloves, which might have resulted in greater number of shoots. Meristem culture in garlic gave successful results for the establishment of plants (Messiaen *et al.* 1993), where plantlets were successfully obtained. With successive shoot formation from explant meristem size ranged from 0.4 to 0.6 mm with the 87% of plants were virus free (Havranek and Novak 1973), virus free garlic plants were produced through shoot tip culture (Peña-Iglesias and Ayuso 1982). For initiation of plant growth. The best medium containing BA, IBA, and GA through meristem tip culture (Ghosh *et al.* 1997). Thermotherapy followed by meristem tip culture produced virus free garlic plants (Bruna *et al.* 1997; Manjunathagowda *et al*. 2021). The phenyl derivatives TDZ and N-phenyl-N'-(-2-chloro-4-pyridyl) urea (4PU-30), were added to the B5 and MS medium for subcultures to increase for the micro-propagation of garlic viz. proliferation rate of garlic cultivar (Rossi *et al.* 1995).

The multiple shoot production is most important to produce the maximum number of propagules. A meristem may develop either into single shoot or multiple shoots and plantlets (Bajaj and Dhanju 1981). A high cytokinin concentration is utilized to overcome the apical dominance of shoot and enhance the branching of lateral buds from leaf axils, BA was the most effective cytokinin for stimulating auxiliary shoot proliferation, followed by kinetin and 2-ip (Kitto and Young 1981; Papachatzi *et al.* 1981). Endogenous auxins do not promote auxiliary proliferation; however, their presence may improve culture growth (Wang and Hu 1980). One of the possible roles of auxin in stage II medium is to nullify the suppressive effect of high cytokinin concentration on auxiliary shoot elongation and restore normal shoot growth. The highest TDZ concentration increases the number of shoot number but decreases in shoot size, although rooting was normal (Bertaccini *et al.* 2004), thus it aids in the regeneration and proliferation of meristems into shoots.

## Conclusion

The study inferred that, the survival percentage, initiation and multiplication of meristem tip in MS medium supplemented with TDZ (1.0 mg/L) with NAA (0.15 mg/L), TDZ (1.0 mg/L) with NAA (0.05 mg/L) and MS medium supplemented with TDZ (0.5 mg/L) with NAA (0.05 mg/L) gave better plant growth with a greater number of multiple shoots from all three varieties (G-41, G-282, G-323). Hence, these protocols can be utilized for meristem tip culture of garlic for the production of virus free planting material.

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## Author Contribution

Contribution to this paper by each author be given here.

## Conflicts of Interest

The authors declare no conflict of interest.

## Data Availability

The text and Supplementary Materials provide the data that was used to accomplish the study goal. On request from the relevant author, raw data used in statistical analyses is accessible for future usage.

## Ethics Approval

Not applicable in this paper

## References

Ayabe M, S Sumi (2001). Mass multiplication of shots through shoot tip culture of garlic. RDA. *J Hortic Sci* 40:14–19

Bajaj YPS, MS Dhanju (1981). Regeneration of plants from callus cultures of napier grass (*Pennisetum purpureum*). *Plant Sci Lett* 20:343–345

Barandiaran X, A Di Pietro, J Martin (1998). Biolistic transfer and expression of a uidA reporter gene in different tissues of *Allium sativum* L. *Plant Cell Rep* 17:737–741

Bertaccini À, S Botti, D Tabanelli, G Dradi, C Fogher, A Previati, F Da Re (2004). Micropropogation and establishment of mite-borne virus-free garlic. *Acta Hortic* 631:201–206

Bruna A, JL Burba, Galmarini (1997). Effect of thermotherapy and meristem tip culture on production of virus free garlic in Chile. *Acta Hortic* 433:631–634

Ghosh DK, YS Ahlowat, Gupta (1997). Production of virus free plants by thermotherapy and meristem tip culture. *Ind J Agric Sci* 67:591–593

Haque MS, T Wada, K Hattori, L Plas (1997). Garlic roots for micro propagation through *in vitro* bulb let formation. *Acta Hortic* 520:45–52

Havranek P, FJ Novak (1973). The bud formation in the callus cultures of *Allium sativum* L. *Zeitschrift für Pflanzenphysiologie* 68:308–318

Kitto SL, MJ Young (1981). *In-vitro* propagation of Carrizo citrange. *HortScience* 16:305–306

Manjunathagowda DC, J Gopal, R Archana, KR Asiya (2017). Virus-free seed production of garlic (*Allium sativum* L): Status and prospects. *Intl J Curr Microbiol Appl Sci* 6:2446–2656

Manjunathagowda DC, K Jayaswall, M Singh, R Sagar, P Chaturvedi (2021). Thermotherapy of cloves for *in-vitro* mericlone production for healthy and sustainable management of garlic germplasm. *Ind J Tradit Knowl* 20:262–266

Messiaen CM, H Lot, B Delecolle (1993). Thirty years of France experience in the production of disease-free garlic and shallot mother bulbs. *Proc Intl Symp Alliums Trop* 358:275–280

Murashige T, F Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15:473–497

Nagakubo T, A Nagasawa, H Ohkawa (1991). Micro propagation of garlic through *in vitro* bulblet formation. *Plant Cell Tiss Org Cult* 32:175–183

Peña-Iglesias A, P Ayuso (1982). Characterisation of Spanish garlic viruses and their elimination by in vitro shoot apex culture. *In: IV Conf Recent Adv Veg Virus Res* 127:183–193

Papachatzi M, PA Hammer, PM Hasegawa (1981). *In vitro* propagation of Hosta decorata'Thomas Hogg'using cultured shoot tips. *J Amer Soc Hortic Sci* 106:232–236

Quak F (1977). Meristem culture and virus free plants. *In: Applied and fundamental aspects of Plant Cell Tissue and Organ Culture*, pp:598–615. Reinert YPS Bajaj (ed.). Springer Berlin, Germany

Rossi V, F Marani, AL Rabiti, N Nits, NE Looney, DJ Nevins, AH Halevy (1995). Phenylurea derivatives for micro propagation of garlic meristems. *Acta Hortic* 394:263–271

Sing (2005). Virus and phytoplasma diseases on onion, garlic and chilli. *In: National Symposium on Current Trends in Onion, Garlic, Chilies and Seed Spices, 25–27 November 2005*. Rajgurunagar, Pune, India

Wang PJ, NY Hu (1980). Regeneration of virus free plants through in vitro culture. *Advances in Biochemical Engineering: Plant Cell Culture*. *II*. Springer, Berlin, Germany, pp:61–99