Embryogenesis and plantlet formation in garlic *Allium sativum* L.

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ABSTRACT

An *in vitro* method for propagation of garlic (*Allium sativum*) *via* somatic embryogenesis is described. Complete plantlets were obtained by a sequential supply of different basal media and growth hormones. Murashige & Skoog's medium with kinetin (5.0 ppm) and 2,4-dichlorophenoxy acetic acid (0.5 ppm) promoted embryogenic callus formation. Shoot regeneration was achieved by transferring embryogenic callus to Gamborg's B_5 medium with kinetin and indole acetic acid whereas root formation occurred in same basal medium supplemented with α-napthalene acetic acid (0.1 ppm). Sucrose at a higher concentration (10%) had stimulatory effect on shoot regeneration potential of embryoids. A characteristic garlic odour developed in this culture.

Key words: *Allium sativum*, garlic, micropropagation.

Abbreviations

B_5 : Gamborg's medium
2,4-D : 2,4-dichlorophenoxy acetic acid
IAA : Indole acetic acid
Kn : Kinetin
MS : Murashige & Skoog's medium
NAA : α-napthalene acetic acid

Garlic (*Allium sativum* L.) besides being used in the household is also an important ingredient in indigenous systems of medicines. Owing to sterility of all genotypes, garlic breeding is restricted to the selection of spontaneous mutations and their vegetative propagation. *In vitro* techniques of rapid propagation have been tried as nonconventional breeding methods for garlic (Novak 1984; Novak, Havel & Dolevzel 1986; Rauber & Grunewaldt 1988; Nagasawa & Finer 1988). The regeneration process, reported in garlic so far involves shoot differentiation (Novak, Havel & Dolervzel 1986). Somatic embryogensis was earlier reported by Abo-El-Nil (1977) in garlic, but plant regeneration is till undefined.

Surface sterilization of cleaned bulblets of garlic was done by 70% (v/v) ethanol for 2 min followed by 0.1% $HgCl_2$ (w/v)

for 5 min and subsequent rinsing in sterile distilled water. The meristmatic shoots were removed with a sharp razor and inoculated in MS medium containing combinations of BA, Kn and 2, 4-D. The ratio of cytokinins (BA and Kn) and 2,4-D was kept constant at 10:1 and 5:1, respectively. The cultures were maintained at 25 ± 2°C in 16 h light and 8h dark regime.

The response and nature of callus tissue produced from shoot meristems is presented in Table 1. Better callus initiation took place after a month in static MS medium containing 5.0 ppm Kn with 0.5 ppm 2, 4-D. An initially pale, nodular, slow growing callus turned brown in next subculture, after 10 days, in the same medium but 2,4-D alone (0.1 ppm) was suitable for maintenance of cultures (Fig. 1a). Microscopic examination of the callus revealed that 90 per cent of the tissue was comprised of embryoids of varying sizes. Embryoid development was non synchronous. Embryoids of various shapes and sizes were observed in a single flask (Fig. 1 b & c). For further development of the embryoids, three basal media viz., MS (Murashige and Skoog's 1962), B_5 (Gamborg, Miller & Ojima 1986), and White's (1953) were tried, out of which only B_5 promoted differentiation. Combinations of IAA (1.0ppm) + Kn (0.5 ppm) favoured shoot differentiation (Fig. 1d). The developed shoots when transferred to NAA (0.1 ppm) alone formed roots and a small bulb was also formed when kept for longer period (8 weeks) in the same medium (Fig. 1 e).

Havranek & Novak (1973) first reported callus establishment in garlic by culturing young leaves of garlic on MS medium supplemented with Kn (9.3 μM) and 2,4-D (4.5 μM). However, this callus was not embryogenic. Later, Abo-El-Nil (1977) cultured stem explants in AZ medium with chloro-phenoxy acetic acid, 2,4-D and Kn and obtained embryogenic callus. The embryo forma-

Table 1. Morphogenetic response of *Allium sativum* cultures

Kn + 2,4-D (ppm)	Response percentage (callus/shoots/roots)	Morphogenetic nature of tissue formed
1.0 + 0.1	90	CC
2.0 + 0.2	95	CC
5.0 + 0.5	100	EC
1.0 + 0.2	75	SC
2.0 + 0.4	60	NC
5.0 + 1.0	80	SC
0.1 + 0.01	—	—
0.2 + 0.02	—	—
0.5 + 0.05	25	R
1.0 + 0.01	80	SC

CC- compact callus; NC- nodular callus; SC- semifriable callus; R- roots; EC- embryogenic callus

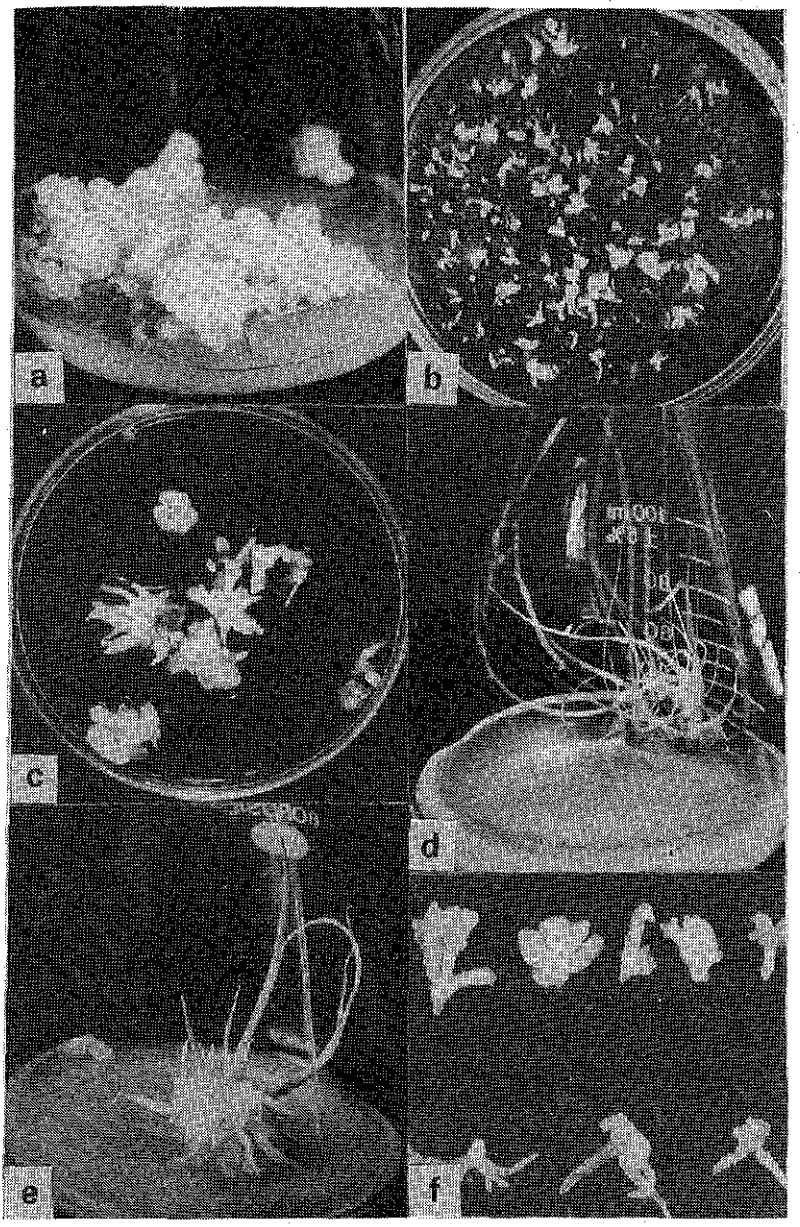

Fig. 1. Micropropagation of *Allium sativum*

a. Embryogenic callus on MS medium with 2, 4-D (0.1 ppm) b. & c. Embryoids of various sizes and shapes growing on MS medium with 2, 4-D (0.1 ppm) for 4 weeks d. Multiple shoots on B_5 medium with Kn (0.5 ppm) and IAA (1.0 ppm) after 6 weeks e. Root and bulb differentiation in B_5 medium with Kn (0.1 ppm) after 8 weeks f. Upper row : Shoot differentiation in high sucrose Lower row : Root differentiation in low sucrose

tion was sporadic and these developed only till torpedo stage. In all previous reports, plantlets were regenerated through shoot bud differentiation (Abo-El-Nil 1977; Novak, Hevel & Dolervzel 1986; Rauber & Grunewaldt 1988; Nagaswa & Finer 1988) and 2,4-D and Kn were found neccessary for induction of callus. Micropropagation of garlic *via* embryogenesis has been achieved in the present investigation. The source of explant seems to play an important role in determining the morphological nature of callus produced (embryogenic or non-embryogenic). Although 80 to 90 per cent of tissues formed somatic embryoids on MS medium, they did not sprout further on the same medium. Shoot and root development was observed when embryogenic callus was transferred to B_5 basal medium, which contain lower concentration of salts as compared to MS medium. Lower concentration of salts may have favoured sprouting of embryoids. Among the various other adjuvants employed for enhancing the frequency of embryo development and their sprouting, lower sucrose (2%) produced higher number of embryoids whereas, higher sucrose (10%) markedly influenced shoot development (Table 2 & Fig. 1 f). The importance of higher sucrose in the formation of embryogenic callus was demonstrated earlier by Lu, Vasil & Ozias-Akins (1982) in *Zea mays*. On the contrary, Amimirato & Steward (1971) in *Daucus* sp. and *Sium suave* and Leonor, Pedro & Juan (1988) in *Digitalis obscura* observed inhibitory effects of high sucrose levels. According to Rauber & Grunewaldt (1988), the efficiency of existing *in vitro* techniques which is through shoot bud differentiation seems to be of no value in *Allium* breeding programmes because of its slow multiplication rate. The present method not only can be applied to conserve non flowering breeding material but also can be used for rapid multiplication of the selected clone.

Table 2. Influence of sucrose on frequency of embryoid formation and differentiation in *Allium sativum*

Sucrose (%)	No. of embryoids /gram of tissue	Shoot development (%)
0	—	—
2	105	—
4	80	—
6	53	20
10	52	80
12	21	15

References

Abo-El - Nil M M 1977 Organogenesis and embryogenesis in callus cultures of garlic (*Allium sativum* L.). Plant Sci. Lett. 9 : 259-264.

Ammirate P V & Steward F C 1971 Some effects of environment on the development of embryos from cultured free cells. Bot. Gaz. 132 : 149-158.

Gamborg O L, Miller R A & Ojima K 1986 Nutrient requirements of suspension cultures of soyabean root cells. Exp. Cell Res. 50 : 151-158.

Havranek P & Novak F J 1973 The bud formation in the callus culture of *Allium sativum* L. Z. Pflanzenphsiol. 68 : 308-318.

Leonor L, Pedro P B & Juan S 1988 Morphogenesis in hypocotyl cultures of *Digitalis obscura* - influence of carbohydrate levels and sources. Plant Science 57 : 247-252.

Lu C, Vasil I K & Ozias-Akins P 1982 Somatic embryogenesis in *Zea mays* L. Theor. Appl. Genet. 62 : 109-112.

Murashige T & Skoog F 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15 : 473-497.

Nagasawa A & Finer J J 1988 Development of morphogenic suspension cultures of garlic (*Allium sativum* L.). Plant cell, tissue and organ culture 15 : 183-187.

Novak F J 1984 Somaclonal variation in garlic tissue culture as a new breeding system. Eucarpia 3rd *Allium* Symposium, 4-6 September 1984 (pp. 39-43). Institute for Horticultural Plant Breeding (IVT), Wageningen, The Netherlands.

Novak F J, Hevel L & Dolervel J 1986 *Allium*. In: Evans, Sharp & Ammirato (Eds.) Handbook of Plant Cell Culture Vol. 4. (pp. 419-456). Macmillan Publishing Company.

Rauber M & Grunewaldt J 1988. *In vitro* regeneration of *Allium* species. Plant Cell Reports 7 : 426-429.

White P R 1953 A comparison of certain procedures for maintenance of plant tissue cultures. Am. J. Bot., 40 : 517-524.