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RESEARCH ARTICLE

EFFECT OF 2,4-D AND NAA ON SOMATIC EMBRYOGENESIS FROM APICAL PORTION OF GROUNDNUT (*ARACHIS HYPOGAEA* L.)

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Abstract

Somatic embryogenesis was carried out epicotyl portion of the mature embryo/apical portion. The somatic embryo induction medium containing 2,4-D or NAA (10.0 to 50.0 mg/l). Of the two concentrations tested 2,4-D (30.0mg/l) recorded the highest percentage of response followed by NAA (30.0mg/l). But the highest number of somatic embryo were recorded in 30.0mg/l of 2,4-D followed by NAA. The apical portion of the mature embryo formed direct embryos without any intervention of callus. The maximum percentage of embryogenic cultures were noticed in 30.0mg/l of 2,4-D followed by NAA at 30.0mg/l. for the differentiation of somatic embryos, the embryogenic masses were transferred to medium without any growth regulator. The maximum number of somatic embryos per culture was recorded in 30 mg/l of 2,4-D followed by 30.0 mg/l of NAA.

Keywords: *Arachis hypogaea* L.,Somatic Embryogenesis, 2,4-D and NAA

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Introduction

Somatic embryogenesis is preferred over plant multiplication because is transformation and proliferation potential is very high and the occurrence of chimeric plants can be minimized significantly (Stefaniak, 1994). Plantlet regeneration has been

possible through somatic embryogenesis, organogenesis and callus cultures (Changalrayan *et al*., 1997). Plant regeneration *via* somatic embryogenesis occurs in a wide number of plant species including some species of *Arachis* such as *A.hypogaea* (Sellars *et al*., 1990; Baker and Wetzstein, 1992; Durham and Parrott, 1992).

In vitro regeneration of plants *via* somatic embryogenesis promises a higher potential for use in plant propagation and gene transfer (Ammirato, 1984; Parrot *et al*., 1991a). Peanut improvement through biotechnology will largely depend on efficient regeneration system. Plant regeneration via somatic embryogenesis has been reported from mature embryo axes (Mckently *et al*., 1991), immature zygotic embryos (Hazra *et al*., 1987; Ozias-Akins *et al*., 1992) and immature leaf (Sabitharani and Reddy, 1996).

Direct somatic embryogenesis from immature zygotic embryos and 50% conversion was reported in groundnut by (Hazra *et al*., 1989 and Ozias-Akins, 1989) indicated problems on conversion of somatic embryos into plantlets. Somatic embryogenesis for the most part happens by implication by means of callus stage or straightforwardly from early explants.. Among the various modes of plant regeneration from *in vitro* cultures, somatic embryogenesis has emerged as most efficient and promising tool for peanut (*Arachis hypogaea* L.) crop improvement programmes (Mckently, 1995).

Materials method

Mature, healthy and uniform seeds of groundnut variety TMV-7 was obtained from Tamil Nadu Agricultural University, Coimbatore and used as an experimental material to carry out the somatic embryogenesis.

The epicotyledonary portion /apical portion of the mature embryos were used as an explant source for somatic embryogenesis. The apical portion of mature embryos were placed on solid somatic embryo induction medium composed of MS salts, B5 vitamins, 3% sucrose and 8 % agar agar supplemented with varying concentrations of 2,4-D and NAA (10.0 to 50.0 mg/l) pH was adjusted to 5.8 with 0.1 NaOH and 0.1 N HCL prier to autoclaving at 120o C for 15 minutes. After thirty days of inoculation, the responsive cultures were transferred to MS basal medium for maturation of somatic embryos and subsequently cultured on plantlet growth medium containing BAP or KIN.

Results

The direct somatic embryogenesis were observed using auxins alone. The addition of cytokinins like BAP or KIN did not influence either the percentage of embryogenic cultures nor number of somatic embryos per culture. The somatic embryos were induced only in solid medium.

The apical portion of the mature embryos were cultured on somatic embryo induction medium containing 2,4-D and NAA individually. The auxin concentrations was ranged from 10.0 to 50.0mg/l. The apical portion of the mature embryos directly formed somatic embryos without intermediate callus phase when inoculated on auxin. Values with the same superscript are not significantly different at the 0.5% probability level according to Duncan‟s Multiple Range Test.

Containing medium the apical portion of the mature embryos were cultured on somatic embryo induction medium with different concentrations of NAA. The percentage of response and mean number of somatic embryos per culture was increased upto 30.0mg/l of NAA and then there was a reduction in responsive culture and number of somatic embryos per culture. The same trend was also noticed in varying concentrations of 2,4-D. Among the various concentrations of 2,4-D 30.0 mg/l recorded maximum percentage of response and mean number of somatic embryos.Based on observation in terms of somatic embryogenesis 2,4-D was better than NAA. (Table-1, Plate-1).

Table:1.Effect of NAA and 2,4-D on Somatic embryogenesis from embryonal leaflets

axes in groundnut Cv.TMV-7 (Mean ±SD) Hormone mg/l		Percentage of responsive explants	Mean number of somatic embryos/ culture
NAA	10	72.24±1.10bc	5.20±0.39cd
	20	75.33±1.33ab	6.20±0.75bc
	30	78.12±1.24 a	12.40±0.84a
	40	67.10±0.98 d	6.80±1.34 b
	50	55.80±1.04e	4.20±0.43 de
2,4-D	10	73.44±1.01cd	7.10±0.75 de
	20	77.33±1.14ab	11.80±0.50 b
	30	80.24±1.20 a	15.10±0.75 a
	40	74.00±1.44cb	10.20±1.38bc
	50	65.54±0.84e	8.00±0.48d

Plate 1: Somatic embryogenesis from apical portion of the whole embryonal axix of groundnut Cv TMV-7

a. Somatic embryo initiation from apical portion
b. Development of somatic embryos
c & d. Fully developed somatic embryos
e. Plantlet transferred to plastic cup

Discussion

Our study showed that, the production of embryogenic cultures and number of somatic mbryos per culture/explants depend upon the strength of the auxin in the induction medium. Among the different concentrations of auxins, 2,4-D and NAA were used for somatic embryrogenesis in groundnut. In particular 2,4-D was more effective than NAA. A similar study has been carried out in groundnut Hazra *et al*., (1989) and in soybean Lazzeri *et al*., (1987).

The present research work clearly demonstrated that, NAA was comparatively less effective in inducing somatic embryogenic cultures.There was no secondary production in NAA induction medium. The same was reported by Mckently, (1991) who found that NAA was less effective than picloram or 2,4-D in inducing embryogenic cultures. In contrast, Sellars *et al*., (1990) found that a constant application of 2.0mg/1of NAA resulted in high production of embryos from immature embryos of peanut. Like wise Ozias-Akins, (1989) obtained smooth nodular out growths or neomorphic protuberances on immature zygotic peanut cotyledons using 5.0-30.0 mg/1 of NAA.

The results of the present study clearly indicated that either 2,4-D or NAA at higher concentrations reduced both the percentage of embryogenic cultures and number of somatic embryos per culture. Baker *et al*., (1994) reported that there was significantly more embryos from immature cotyledonary explants when cultured on mg/1 of 2,4-D in the induction medium. Further, higher concentrations of auxin reduced the somatic embryo production in the embryogenic cultures. Similar studies were reported in tea Bano *et al*., (1991) and papaya Fitch and Mansharot, (1990) where a reduction in embryogenic potential of explants is seen at higher 2,4-D levels. Zhang, (2000) reported that 2,4-D has widely used for the induction of embryogenic callus and embryoids.

When the concentrations of 2,4-D or NAA increased the optimum level, there was problem in obtaining normal embryos. Often 2,4-D produced higher percentage of abnormal embryos when compared to NAA. Mckently (1991) found that when auxin concentrations increased, the probability of obtaining normal shaped groundnut somatic embryos decreased. The influence of the auxin type varied from species to species. According to Hazra *et al*., (1989) no embryo production resulted with NAA in the induction medium. In contrast to this, there are several recent reports using NAA in groundnut Chengalrayan *et al*., (1994); Venkatachalam and Jayabalan, (1996) and in *Cajanus cajan* Mallikarjuna *et al*., (1996). When NAA was used for the induction of somatic embryos, the number of somatic embryos per culture and percentage of responsive explants were low. Even though 2,4-D is a better auxin source for induction of somatic embryogenesis, there is higher possibility for the production of abnormal embryos.

In general, the apical portion of embryo of groundnut requires high concentration of auxin. Sabitharani and Reddy, (1996) on the induction of somatic embryos at very low concentration of 2,4-D ranging from 0.025 to 0.4mg/l. All other reports Ozias-Akins, (1989); Baker and Wetzstein, (1992); Eapen and George, (1993a); Chengalrayan *et al*., (1994); Mckently, (1995), Baker *et al*., (1995), Venkatachalam and Jayabalan, (1997) were in conformity with our results.

Conclusion

The present findings may be useful for regeneration of plantlets from somatic embryos on simple medium might be used for the production of somaclones of plants and for the storage and the maintenance of germplasm.

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