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BRIEF COMMUNICATION

Evolution of ethylene and methane in relation to somatic embryogenesis in chickpea

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Abstract

In four genotypes of chickpea (*Cicer arietinum* L.) BG 362, BG 372, BG 329 and C235 the relationship between somatic embryogenesis of leaf explants and ethylene and methane evolution was studied. In BG 362, which was more embryogenic than other genotypes, a higher ethylene:methane ratio of 5.8:1 at day one after inoculation in the induction medium and a lower ethylene:methane ratio of 2.89:1 in the maturation medium was found. On the contrary, in BG 372 with the least embryogenic potential, a lower ethylene:methane ratio of 1.7:1 in the induction medium and a higher ethylene:methane ratio of 4:1 in the maturation medium was found. Thus, these ratios in induction and maturation stages seems to be markers for embryogenesis in leaf explants of chickpea.

Additional key words: *Cicer arietinum*, induction stage, maturation stage.

Ethylene evolved in culture vessels plays a major role in the morphogenesis of cells and tissues cultured *in vitro*. Ethylene evolution in *in vitro* cultures is related to embryogenesis and it was reported that in some plant species, there is an absolute need for an optimum level of ethylene for embryogenesis (Cho and Kasha 1989). On the contrary, inhibition of shoot regeneration from callus cultures by ethylene was reported in *Helianthus annuus* (Robinson and Adams 1987) and in *Nicotiana*

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Abbreviations: E:M ratio - ethylene:methane ratio; DAI - days after inoculation; BAP - benzylaminopurine; Kn - kinetin; Gln - glutamine; IBA - indolebutyric acid; 2,4-D - 2,4-dichlorophenoxyacetic acid.

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tabacum (Huxter *et al.* 1981). Addition of silver nitrate, an inhibitor of ethylene, to the media increased shoot regeneration from callus cultures in *Triticum aestivum* (Purnhauser *et al.* 1987) and caused enhanced embryogenesis in *Daucus carrota* (Roustan *et al.* 1990), *Brassica campestris* var. *gemmifera* (Williams *et al.* 1990), *Brassica campestris* ssp. *pekinensis* and other recalcitrant *Brassica* genotypes (Chi *et al.* 1991, Palmer 1992, Pua *et al.* 1996).

In chickpea, a certain amount of ethylene is reported to be necessary for embryogenesis, and the optimum level depends upon the type of explant, genotype and stage of the culture (Patil *et al.* 1998). Along with ethylene, certain amount of methane is also evolved in the culture vessels. But so far, there is no report on the role of methane in morphogenesis of cells and tissues in culture. Studies of Babbar and Gupta (1986) suggested that the ethylene precursor S-adenosyl-methionine enhanced embryo formation in *Datura metel*. Addition of 0.01 % methyl methane sulphonate enhanced multiple shoot formation in cotyledon explants of *Cucumis sativus* (Mustafa *et al.* 1991). Direct evidence on the role of methane in organogenesis of different explants of chickpea grown *in vitro* was reported by Chandra *et al.* (1997/98).

The purpose of the present study is to assess the role of ethylene, methane and also the ethylene:methane (E:M) ratio in relation to the embryogenic potential of leaf explants of four genotypes of chickpea.

Four genotypes of chickpea (*Cicer arietinum* L.) C 235, DG 362, DG 329 and BG 372 were obtained from the Division of Genetics, Indian Agricultural Research Institute, New Delhi. Seed germination, media preparation and inoculation of explants were carried out under aseptic conditions according to Chandra *et al.* (1993). Leaf segments (3 mm^2) from 13-d-old seedlings were used as explants. The induction medium consisted of Murashige and Skoog (MS) medium supplemented with B5 vitamins to which 1.25 mg dm^{-3} 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.25 mg dm^{-3} kinetin (Kn) were added. After growing them for 45 d in the induction medium, the embryogenic calli were transferred to the embryo maturation medium which was composed of MS medium suplemented with benzylaminopurine (BAP; 2 mg dm^{-3}), Kn (1 mg dm^{-3}), indolebutyric acid (IBA; 1.25 mg dm^{-3}), glutamine (Gln; 1315 mg dm^{-3}) and mannitol (3 %).

Observations on growth and morphogenesis were recorded at weekly intervals. Data on the evolution of ethylene and methane were recorded 1 and 30 d after inoculation (DAI) in the induction medium and 1 d after inoculation in the maturation medium. For the measurement of ethylene and methane, the culture tubes were capped with serum stoppers after removing the cotton plugs and incubated for 24 h. Ethylene and methane were measured in gas samples (2 cm^3) taken out from tubes with the help of a disposable syringe and injected into the gas chromatograph (*Perkin-Elmer, Sigma 2000* model, USA) fitted with flame ionisation detector. For each treatment, 4 - 6 tubes were used and the measurements were replicated four times. Detection of both the gases was confirmed with the help of standards.

Data were statistically analysed using the factorial completely randomized design and the significance of differences was evaluated at $P = 0.05$ using Duncans' multiple range test.

Amongst the genotypes, BG 362 produced somatic embryos which later developed into fully developed embryos in the maturation medium. Though the genotype C 235 produced embryoids, they failed to develop fully. Genotypes BG 372 and BG 329 remained as non-embryogenic calli.

Evolution of ethylene and methane was observed at 1 and 30 DAI in induction medium (Fig. 1*A*,*B*) and at 1 DAI in the maturation medium (Fig. 1*C*). Of all the genotypes studied, BG 362 which produced somatic embryos recorded the highest evolution of ethylene at 1 DAI in the induction medium followed by genotype C 235 (Fig. 1*A*). At 1 DAI, the methane evolution was low in BG 362. Thus, a maximum E:M ratio of 5.8:1 was recorded in this genotype followed by genotype C 235 which also produced embryoids in the induction medium. At 30 DAI, the evolution of both ethylene and methane declined in BG 362 (Fig. 1*B*), but more in ethylene, resulting in a lesser E:M ratio of 1.7:1. In this genotype, the ethylene evolution in the maturation medium at 1 DAI was lesser and the methane evolution was higher

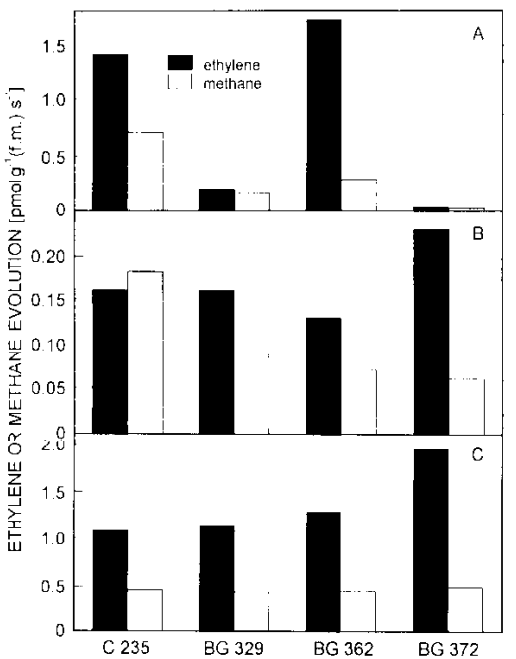

Fig. 1. Evolution of ethylene and methane in four genotypes of chick pea at 1 DAI in induction medium (*A*), 30 DAI in induction medium (*B*), and 1 DAI in maturation medium (*C*). Means of 4 repetitions.

as compared to that at 1 DAI in the induction medium (Fig. 1*C*) resulting in an E:M ratio of 2.89:1. Closely following this genotype was C 235 which recorded an E:M ratio of 2:1 at 1 DAI and 0.9:1 at 30 DAI in the induction medium and 2.37:1 at 1 DAI in the maturation medium. The number of embryoids induced and matured

into somatic embryos was lesser in this genotype as compared to BG 362.

The other two chickpea genotypes BG 372 and BG 329 produced non-embryogenic calli. The amount of ethylene recorded in these two genotypes at 1 DAI in the induction media was lesser than that of BG 362 and C 235 (Fig. 1*A*). In BG 372, the E:M ratio was 1.7:1 at 1 DAI and 4:1 at 30 DAI in the induction medium and 1 DAI in the maturation medium. At 1 DAI in the maturation medium, ethylene evolution in BG 372 was significantly higher than in other genotypes (Fig. 1*C*). Genotype BG 329 recorded an E:M ratio of 1.2:1 at 1 DAI and 1.8:1 at 30 DAI in the induction medium. It can be seen that the trend of E:M ratio in these two genotypes is just the reverse of the other two embryogenic genotypes. The growth of callus (fresh mass) was highest in the genotype BG 329 (data not shown).

In embryogenic studies, it is a routine practice to add higher concentrations of auxin in the induction media and the most commonly used auxin is 2,4-D (*e.g.* Lazzeri *et al.* 1988). The presence of 2,4-D in the media resulting in an increase in the evolution of ethylene has been reported by many workers (*e.g.* Garcia and Einset 1983, Purnhauser *et al.* 1987). Patil *et al.* (1998) in chickpea and Cho and Kasha (1989) and Evans and Batty (1994) in barley reported that the maintenance of a particular level of ethylene is essential for embryogenesis. Patil *et al.* (1998) also found a linear relationship between inoculation time and evolution of ethylene upto 24 h. On the other hand, Khehra and Mathias (1992) and Tisserat and Murashige (1977) had reported that the evolution of ethylene in the presence of 2,4-D did not influence embryogenesis.

The type and age of explants and the growth regulators in the medium play an important role in the evolution of ethylene which in turn influences the differentiation of the explants (Chandra *et al.* 1997/98). From our results, it is evident that initially increased evolution of ethylene in the induction media followed by its drop in the maturation media helped BG 362 to produce viable embryoids. Addition of silver nitrate ($AgNO_3$), an inhibitor of ethylene, to the induction medium resulted in the formation of non-embryogenic calli in chickpea (Patil *et al.* 1998). Besides the role of ethylene, the present study also supports the role of methane in embryogenesis. In BG 362, low E:M ratio at 30 DAI in induction medium and at 1 DAI in maturation medium was accompanied by embryo maturation, while high E:M ratio at initial stages of induction was accompanied by embryo induction. On the other hand, the genotype BG 372 which produced non-embryogenic callus, recorded significantly lower E:M ratio at 1 DAI in the induction medium as compared to BG 362. Miller and Pengelly (1984) reported that in *Nicotiana* and *Lycopersicon*, cell lines exhibiting low ethylene evolution upto 15 DAI developed unorganised tissue, whereas those with higher evolution of ethylene produced shoots. Similarly in *Pinus radiata* increased evolution of ethylene upto 10 to 15 DAI promoted shoot-bud formation in *in vitro* grown cotyledonary tissues whereas, excessive accumulation of ethylene after initiation of buds resulted in callus formation (Kumar *et al.* 1987).

The role of methane which is also evolved along with ethylene and CO_2 in *in vitro* cultures was rarely reported. In sugar beet, habituated organogenic lines preferentially synthesized methyl, methenyl or formyl groups from S-adenosyl-methionine, whereas polyamine pathway predominated in habituated non-

organogenic lines (Hagege *et al.* 1994). In cotyledon cultures of cucumber, application of methylmethane sulphonate induced multiple shoot formation (Mustafa *et al.* 1991). Also in pigeon pea the role of methane in induction and elongation of shoot buds from cotyledonary node explants was established (Sudarsana Rao 1997). Application of ethrel to the media increased ethylene evolution and reduced the evolution of methane resulting in the production of only non-embryogenic callus (Sudarsana Rao 1997). In addition to the role of ethylene and methane, the present study also helps in understanding the role of E:M ratio in embryogenesis.

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